PHYSICAL PROPERTIES OF LACCASE-MEDIATOR DELIGNIFIED PULPS

A Dissertation Submitted by

Kaaren K. Haynes

B.S. Paper Science and Engineering, University of Washington
M.S. Helsinki University of Technology, Helsinki, Finland

in partial fulfillment of the requirements of the
Institute of Paper Science and Technology
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LIST OF SYMBOLS

Mediator Symbols

ABTS	2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
HTI	N-hydroxypthalimide
HBT	N-Hydroxybenzotriazole
NHAA	H-acetyl-N-phenylhydroxylamine (chemical name) or
NHAA	N-hydroxyacetanilide (common name)
VIO	violuric acid

Bleaching Stages

E	alkaline extraction
O	oxygen delignification
L	laccase only delignification
Lhbt	laccase HBT delignification
Lnhaa	laccase NHAA delignification
Lvio	laccase VIO delignification
A	acetone extraction

Pulp Types

SKP23	loblolly pine kraft pulp with kappa 23
SKP50-1	loblolly pine kraft pulp with kappa 50
SKP50-2	loblolly pine kraft pulp with kappa 50
FBP	fully bleached Douglas fir kraft pulp

Paper Parameters

T	tensile breaking length (length)
Z	zero span tensile (length)
C	fiber coarseness (weight/length)
P	fiber perimeter (length)
l	fiber length (length)
b	fiber-fiber bond strength (N/m^2)
RBA	relative bonded area (unit less)
g	gravitational constant (length/second^2
S_0	scattering coefficient for an unbonded sheet (m^2/kg)
S	scattering coefficient for a paper sheet (m^2/kg)
S	surface charge density (meq/100g)
P	bulk charge density = total charge - surface charge (meq/100g)

Industry Abbreviations

CTMP	Chemical Thermal Mechanical Pulp
MFA	Micro Fibril Angle

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ABSTRACT

This research is a detailed study of the effects of laccase-mediator delignification on the pulp and paper properties of kraft Loblolly pine and fully bleached industrial Douglas-fir kraft pulp. In this study, the mediator N-hydroxybenzotriazole (HBT), and the recently reported mediators H-acetyl-N-phenylhydroxylamine (NHAA) and violuric acid (VIO) are used. Kappa 23 and kappa 50 loblolly-pine kraft pulps and an industrial fully bleached Douglas-fir kraft pulp were used in this research. Comparisons were made between oxygen delignified and the laccase-mediator delignified pulps. The effects of carboxylic acid groups on pulp properties was explored. Surface carboxylic acid groups were measured using ESCA and bulk acid group content was measured using conductometric titration. In addition, the effects of carboxylic acid groups in lignin versus carbohydrates were explored. The hypothesis was that the laccase mediator system could be used to introduce surface acid groups to pulp, and increase the specific bond strength.

Laccase-mediator treatments was selective with respect to lignin. Extractives also played a role and may have effected the selectivity of the process. The laccase-mediator systems introduced carboxylic acid groups exclusively to lignin. The Scott bond strength of laccase-mediator delignified pulps was similar to that of oxygen delignified pulps. When kappa 50 pulp was treated with the laccase-mediator systems, the density of the resulting handsheets was reduced. The presence of lignin or extractives, modified by the laccase-mediator system, negatively impacted paper physical properties. The laccase mediator system had no effect on fully bleached pulp properties above that of a standard alkaline extraction stage. Acid groups in lignin did not increase the specific bond strength.

INTRODUCTION

The delignification of kraft pulps employing enzymatic methods has advanced substantially over the past two decades. In the past, the use of enzymes for delignifying kraft pulps was frequently viewed as an academic pursuit. However, recent advances in biotechnology have suggested that practical applications may occur in the near future. In 1994, Call revealed a laccase mediator system which resulted in up to 50% delignification in only four hours reaction time. This system used laccase and N-hydroxybenzotriazole (N-HBT). In his patent, Call claimed all mediators containing the N-OH group (1). At the June '97 ASWPC conference in Montreal, Amann reported two new mediators: N-hydroxyacetanilide (NHAA), and violuric acid (VIO) (2). These new mediators are considered to be better candidates for mill scale applications because they have a lower nitrogen content, and are more effective at delignification than N-HBT.

Much of the research in this field has focused on the mechanism of reaction, as well as the identification of new mediators. Little work has been done to identify changes in fiber properties after delignification.

Work by Sealey indicated that the laccase mediator system introduced carboxylic acid groups to the residual lignin in kraft pulps (3). Whether or not the laccase-mediator system introduces carboxylic acid groups exclusively to lignin is not known, and is one of the subjects of this research. Carboxylic acid groups on carbohydrates are known to impact paper strength through swelling and increases in specific bond strength (4,5), however, the effect of carboxylic acid groups in lignin has not been thoroughly studied.

This research provides a detailed study of the effects of laccase mediator delignification on fiber properties.

This thesis includes a review of the relevant literature including paper physical properties, paper strength measurement, and bonding. Recent developments in enzyme delignification are also reviewed. Following the literature review, are the research objectives, experimental, results and discussion, and conclusions sections. The results and discussion section is divided into six chapters with conclusions at the end of each chapter, as well as at the end of the thesis document.

LITERATURE REVIEW

FUNDAMENTAL CONCEPTS OF PAPER STRENGTH

Fundamental concepts regarding paper strength are best illustrated using the Page equation. The Page equation is an established prediction of paper strength from fiber and bond properties (6). Equation 1 is the Page equation.

Equation 1

$$\left[\frac{1}{T}\right]=\left[\frac{9}{8Z}\right]+\left[\frac{(12g\cdot C)}{(P\cdot l\cdot b\cdot RBA)}\right]$$

where

T	=	tensile breaking length (length)
Z	=	zero span tensile (length)
C	=	fiber coarseness (weight/length)
P	=	fiber perimeter (length)
l	=	fiber length (length)
b	=	fiber-fiber bond strength (N/m^2)
RBA	=	relative bonded area (unit less)
g	=	gravitational constant -(length/$second^2$)

The limitations of the Page equation are that it is only valid for sheets made with good formation and with straight uncurled or kink-free fibers. This is because sheets with poor formation tend to fail due to high stress at regions of low basis weight, and sheets made from kinked or curled fibers have a lower effective fiber length. Sheet elastic modulus is sensitive to changes in kinks and curls. For this reason, a direct comparison of handsheet tensile strength is only valid when the elastic modulus is constant, indicating no change in kink or curl (7).

In the Page equation, relative bonded area (RBA) is the area of fiber-to-fiber contact in the sheet, and is measured by the use of optical light scattering coefficient (8). In this method,

increasing the wet pressing pressure increases the bonded area. These sheets are then tested for a paper strength property in addition to scattering coefficient. The sheet strength is plotted against scattering coefficient and extrapolated to zero sheet strength. The extrapolated value is the scattering coefficient of unbonded dry fibers, and is used to calculate relative bonded area (8). The relationship between RBA and scattering coefficient is shown in Equation 2 (8).

Equation 2

$$RBA = \frac{(S_0 - S)}{S_0}$$

where S_0 = scattering coefficient for an unbonded sheet (m^2/kg)
S = scattering coefficient for a paper sheet (m^2/kg)

Equation 3

$$\left[\left(\frac{1}{T}\right) - \left(\frac{9}{8Z}\right)\right]^{-1} = b \cdot \left[\frac{1}{\gamma} - \frac{S}{(\gamma \cdot S_0)}\right]$$

where $$\gamma = \left(\frac{12g \cdot C}{P \cdot l}\right)$$

Plotting the Page parameter ($[(1/T) - 9/(8Z)]^{-1}$) versus the scattering coefficient (S) and using Equation 3 allows the bond strength and scattering coefficient of unbonded fibers to be extracted from the slope and intercept of the resulting line. In this manner, the bond strength and RBA of pulps can be calculated through the relatively simple procedure of subjecting handsheets to different degrees of wet pressing.

It has been shown that the absorption coefficient is not always independent of the light scattering coefficient (9-12). The light scattering coefficient is normally measured at 457 nm. Sheets made from unbleached fibers, or sheets containing dye (9, 10), which absorbs light at 457

nm, can exhibit interference in the light scattering coefficient measurement. To correct for this, the scattering coefficient is measured at a higher wavelength (680 nm in this study) at which the lignin material or dye does not absorb light (9,10).

Paper Strength Measurement

The fiber-to-fiber specific bond strength is defined as the bond breaking energy per unit optical area (13). Specific bond strength can be measured directly through the measurement of single fiber crossing, or indirectly calculated based upon strength measurement of the paper sheet (6). In most cases, it is sufficient to measure changes in specific bond strength, and this can be accomplished through measurement of bulk paper properties (14). One paper strength measurement that is considered to be insensitive to fiber kinks or curl is Scott Bond strength (13). The Scott bond test is performed by adhering an elbow shaped metal flange of known area to the paper sheet. The paper sheet is then adhered to a base plate, and a swinging pendulum strikes the flange. The test measures the energy dissipated in pseudo out-of-plane delamination of the test sheet. This test is mildly sensitive to basis weight, but is not sensitive to curl or crimps, since the test measures the adhesion between fibers. The test has been used to measure changes in specific bond strength (5, 13-15), and can be used to compare sheets of differing elastic moduli (5).

Any paper strength measurement is an interaction of many factors, which must be considered when interpreting the measurement. For example, in the finite span tensile test, paper is stressed to failure at a constant rate of elongation. This is accomplished by placing a sample of fixed width between two clamps and stretching it. In the tensile test, the elastic modulus is the

slope of the linear portion of the stress strain curve. As the degree of bonding in the sheet is increased, the tensile strength increases linearly to a certain point, after which the fibers begin to rupture.

Paper is considered bond strength limited in the linear portion of the strength versus relative bonded area curve, when fibers can be pulled intact from the paper network. The fiber strength limited regime is the portion of the strength versus bonding curve which is not linear, and where fibers are ruptured during straining. An illustration of the bond and fiber strength limited regimes is shown in Figure 1. The comparison of strength versus scattering coefficient data must be made within the bond strength limited regime (16).

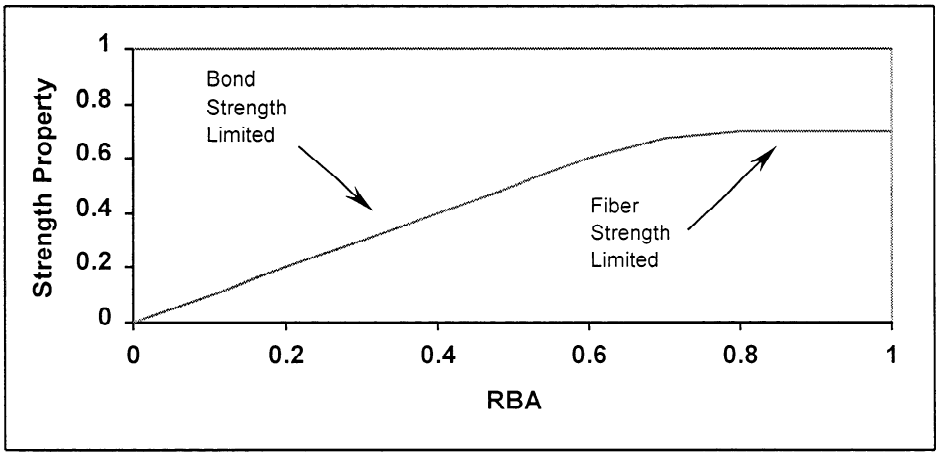

Figure 1: Illustration of bond strength and fiber strength limited regimes (16).

Fiber elastic modulus, a measure of the relationship between stress and strain in the sheet, is dependent on fiber morphology as well as fiber curl and fiber damage. When fibers are curled or damaged, both the tensile strength and elastic modulus are affected.

Elastic modulus can also be measured using non-destructive ultrasonic testing. The ultrasonic elastic modulus is determined by measuring the propagation of longitudinal sound waves between transducer pairs. The in-plane, x or y direction, longitudinal specific stiffness is the elastic stiffness divided by the density ($C_{11}/\rho = V^2$), and is equal to the propagation velocity squared (17). Ultrasonic elastic modulus measurements are higher than mechanical measurements because of the time scale on which the test is made. In mechanical testing of elastic modulus, there is enough time for the sheet to undergo viscoelastic relaxation. In ultrasonic testing there is less time for viscoelastic relaxation, and the ultrasonic stiffness coefficients are always larger then their mechanical counterparts (17).

Effects of Cellulose, Hemicellulose, Lignin and Fines on Bonding

Lignin is a network polymer of phenylpropane units. It occurs primarily in the secondary wall of pulp fibers and in the middle lamella. The type of lignin varies by species and location within the fiber. Up to 70% of the lignin in softwood is located in the secondary wall (19).

Pulps with a high lignin content are more difficult to beat, show poor inter-fiber bonding, and produce sheets with low density and strength values compared to those of lower lignin content. Lignin is relatively inelastic and non-adhesive as compared to hemicellulose, and the presence of lignin restricts swelling and interferes with inter-fiber bonding (20).

Cellulose is the main constituent of wood, and makes up 40-45% of the weight of dry wood, with most of the cellulose located within the secondary wall. Cellulose is a homopolysaccharide composed of β-D-glucopyranose units that are linked together by (β1-4) glycosidic bonds. Cellulose is a two-phase solid made up of amorphous and crystalline regions.

Hydrogen bonding of hydroxyl groups on the surface of cellulose fibrils, both within and between fibers, is generally considered the mechanism of bonding in paper. The polymer properties of cellulose are usually studied in solution, and on the basis of solution properties, conclusions can be drawn about the molecular weight, polydispersity, and chain configuration. Cotton cellulose fibers, for example, have a degree of polymerization (DP) of about 15,000, while wood cellulose had a DP of about 10,000 in its native state (19).

Hemicellulose is the material in wood that is chemically extractable, and has molecular weights of about 180 DP (20). In general, pulps with higher hemicellulose content produce paper with higher strength properties. Hemicellulose is a branched polymer, and increases strength properties by contributing plasticity to the fiber cell wall and by being mobile. Low molecular weight and unhindered molecular chains allow the hydroxyl groups on hemicellulose to find and bond to the hydroxyl groups of the less mobile cellulose surfaces (7).

The effect of fines on bonding cannot be described without qualification concerning the nature of the fines. Fines are generally those materials in pulp that can pass through a 200-mesh screen. Fines that are liberated during beating are generally detached fibrils and surface cellulose material, and act to increase the bonding and tensile strength. Fines may also include lignin or materials that do not bond, and can, therefore, be detrimental to bond strength (13).

Swelling and Carboxylic Acid Groups

Scallan (21) showed that low yield chemical wood pulps act as swellable gels surrounded by semi permeable membranes, and that fiber swelling can be explained by Donnan equilibrium theory. According to the theory, fixed anionic sites in a gel surrounded by a semi permeable

membrane can cause swelling because of the osmotic pressure across the membrane. This osmotic pressure is due to the necessity of electric neutrality. Fixed anionic sites must have counter cations, which cause the concentration of cations inside the membrane to be greater than in the bulk solution. This concentration gradient induces an osmotic pressure, which causes the gel to swell. The gel swells in order to more closely equalize the concentration of metal ions on both sides of the membrane. This situation is shown graphically in Figure 2. The level of swelling will be changed by the number of fixed anionic sites, the concentration of cations in solution, or the charge of the cations.

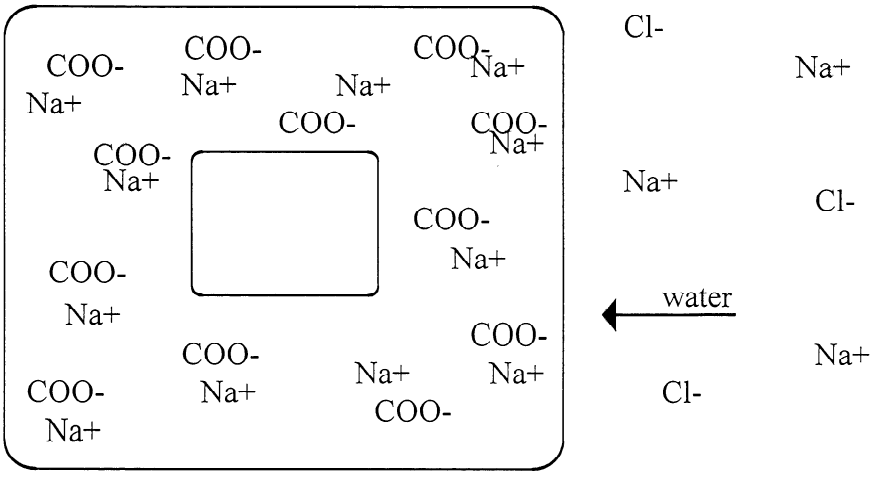

Figure 2: Swelling of a pulp fiber showing concentration gradient induced by bound acidic groups within the cell wall (21).

Within pulp fibers, the carboxylic acid groups provide the fixed anionic sites for metal ions to exchange. All pulps contain carboxylic acid groups with normal ranges of 0.03-0.04 mm/g for fully bleached kraft pulp and 0.07-0.12 mmol/g for unbleached kraft pulp (22). These groups occur naturally in hemicellulose or can be introduced through direct oxidation of

cellulose or lignin. Carboxylic acid groups can be introduced to cellulose fibers in the laboratory by chloroacetic acid treatment (5), and contribute to fiber-fiber bonding (5) and swelling (23).

At least two types of acid groups are believed to exist in pulp fibers (24-26). These acid groups have different equilibrium constants (pKa). The equilibrium constant (Ka) is defined as the concentration of products over reactants at equilibrium. The pKa is the –log of Ka. Acid groups in xylan are believed to be uronic acids with a pKa = 3.3, while carboxylic acid groups in lignin are believed to have a pKa = 5.5. The ability to identify these groups is limited to potentiometric titration, and very little is known about the effects of these different acid groups.

Scallan (27) also researched the area of ion exchange and found that the valence of the counter ion is important to the strength properties of the final sheet of paper. By acid washing the pulp to remove the existing metal ions and then introducing a new metal ion, Scallan discovered that both degree of swelling and paper strength fell with increasing valence of the counter ion. Paper made from ion exchanged pulp has increased strength properties with the order of $Al^{+++}<H^{+}<Mg^{++}<Ca^{++}<Li^{+}<Na^{+}$ (27). Other researchers (28-32) have confirmed this relationship.

Ion exchange with sodium ions results in a pulp with maximum swelling and strength properties. Some of the counter ions present in pulp are closely bound to the molecular chain, and do not contribute to swelling (30). When carboxylic acid groups are converted to the sodium form, dissociation is largely complete, and maximum swelling occurs (33). When multivalent cations are used, the number of ions released into solution is intermediate between the hydrogen and sodium cases, and intermediate swelling results (23).

Temperature and pH have been shown to have an effect on fiber swelling (34,35). In general as temperature drops the fibers swell. Fiber swelling is also affected by pH. In the presence of salt solutions swelling is minimal below pH 3, where carboxylic acid groups are

protonated and do not interact with cations. As pH increases swelling increases until pH 7 is reached, and all carboxylic acid groups are deprotonated. From pH 7 to pH 12, swelling ceases at a constant electrolytic concentration (35).

At very high pH, the concentration of negative ions in solution increases so that many of the counter ions within the pulp become free to balance the charge in solution, and are not bound within the fiber wall. This reduces the concentration gradient and reduces swelling (32). Metal ions in solution, retention aids, and polymers can also affect swelling of pulp fibers by increasing the electrolyte concentration of the solution, and by exchanging with counter ions within the fiber. By determining the number and location of carboxylic acid groups, swelling and ion exchange have been used to understand the sorption of direct dyes, and the retention of polymers (36-40).

Chemical Modification of Fibers

Research has shown that the chemical modification of pulp fibers can influence fiber strength properties, swelling and bonding. Ampulski (41) found an empirical correlation between breaking length, and carboxylic acid group location in CTMP pulp. His correlation is shown in Equation 2, and is dependent on both surface charge and total acidic group content. The R^2 value for this correlation is 0.87. Ampulski speculates that this relationship is due to increased fiber conformity, RBA, and increased specific bond strength.

Equation 4 Breaking length (m) = 103S + 15P + 54

where S = surface charge density (meq/100g)
P = bulk charge density = total charge - surface charge (meq/100g)

Erickson and Sjostrom (42) converted the carboxylic acid groups in high yield sulfite spruce and birch pulps to the ester form with aqueous propylene oxide. This treatment decreased the ability of the pulp to ion exchange, and dramatically reduced swelling and tensile strength. The esterified pulp also had a lower degree of bonding than untreated pulp at the same drainage resistance. The authors speculated that the introduction of hydrophobic groups into the pulp prevented inter-fiber bonding. However, the results are clouded by the fact that the added aliphatic substitution to cellulose would cause all of the observed results.

Xylanases (43,44) and laccases (45,46,47) have been shown to introduce chemical groups to the pulp fibers. Poppius-Levlin et al. (46) studied the effects on pine kraft pulp of laccase alone and in combination with hydroxybenzotriazole (HBT). Laccase alone caused only minor chemical changes to lignin. However, laccase with HBT caused an increase in the carboxylic acid content and a decrease in the free phenolic hydroxyl and methoxyl group contents of the residual lignin.

Sealey (3) found an increase in carboxylic acid groups in residual lignin isolated from softwood brownstock kraft pulp. Some of his results are shown in Figure 3. The lignin was isolated from the pulp fibers and reacted with laccase and the various mediators. It was then chemically labeled, and the carboxylic acid content was measured using ^{31}P NMR. He did not determine whether laccase mediator delignification increases the carboxylic acid content of cellulose or hemicellulose. The terminology in Figure 3 is starting material (SM), laccase only (L), laccase and N-hydroxypthalimide (Lhti), laccase and N-hydroxybenzotriazole (Lhbt), laccase and N-hydroxyacetanilide (Lnhaa), and laccase and violuric acid (Lvio).

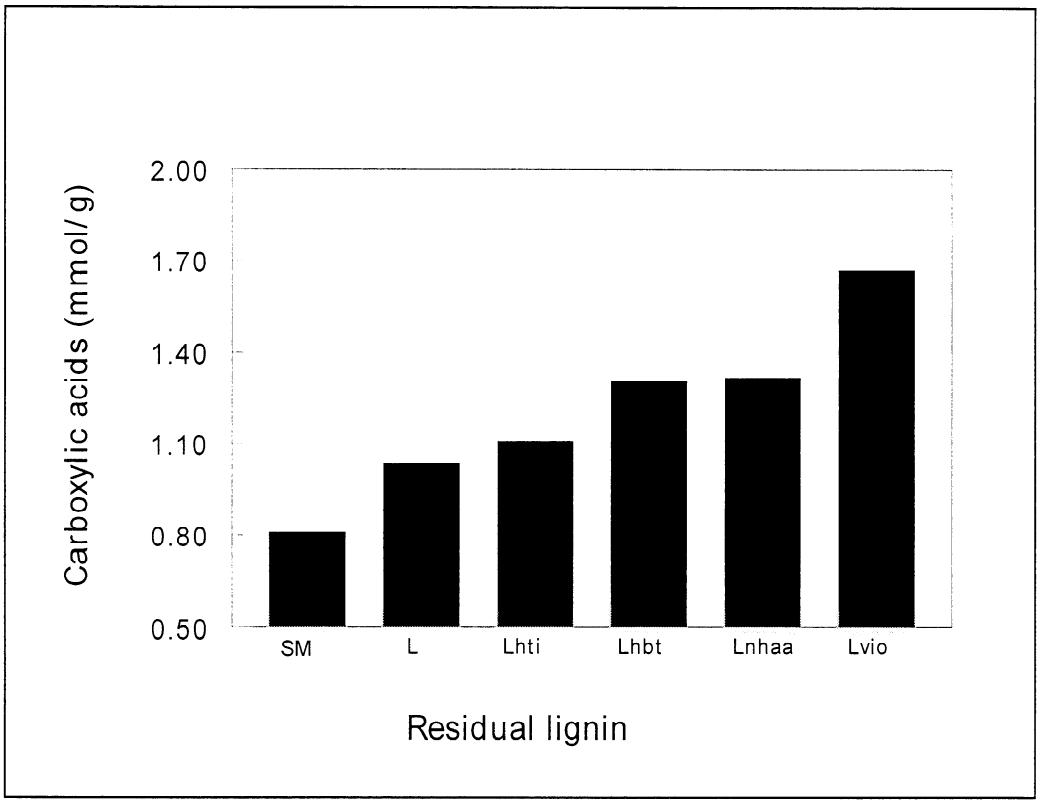

Figure 3: Carboxylic acid concentration by ^{31}P NMR analysis (3) of isolated lignin, reacted with various mediators. Reactions were performed on isolated residual lignin from softwood brownstock kraft pulp. Statistical analysis suggests that any difference greater than 0.18 mmol/g is significant (with 95% confidence).

In recent work by Barzyk (5), a novel method was developed to introduce carboxylic acid groups uniformly across the cell wall of the fiber, or concentrate them on the surface of the fiber. In Barzyk's method, chloroacetic acid substitution occurred with either air-dried or solvent exchange dried fibers. The pulps were first converted to calcium form, and then solvent exchanged or aid dried. The fibers were then treated with a chloroacetic acid solution in water or in solvent, and then washed. The solvent exchanged fibers were then subjected to a restrained drying cycle from water (5). Substitution at the fiber surface was shown to increase the bond strength of paper by as much as 50%. This strength increase is shown in Figure 4.

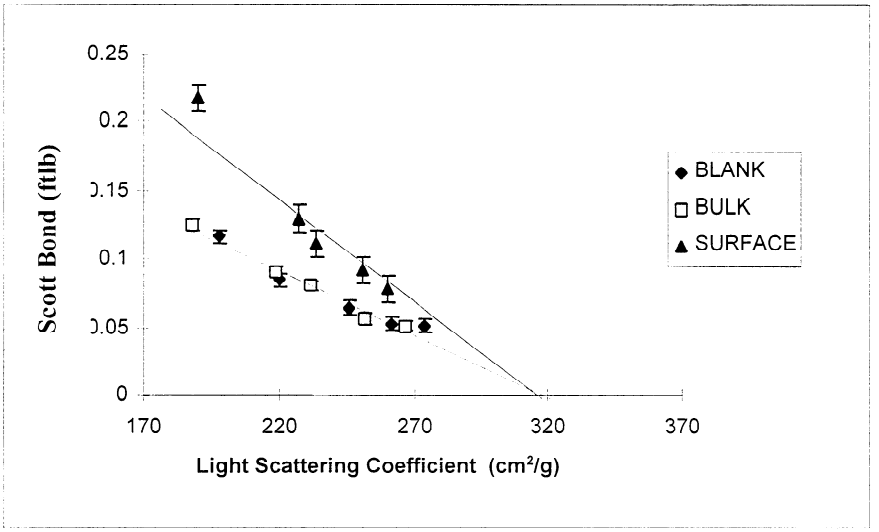

Figure 4: Scott-Bond strength plotted against light scattering coefficient for blank, bulk and surface treated pulps. There is an increase in specific bond strength for surface substituted fibers (5).

Determination of Acid Content

Conductometric titration permits the determination of the number of carboxylic acid groups within a pulp through titration from the hydrogen form (acid pH) with sodium hydroxide. The progress of the titration is measured through conductivity (48).

Scanning Electron Microscopy (SEM) measurements have been used to determine the amount and location of metals within pulp fibers (28), and to discern the structure of viscose fibers (49). To identify carboxylic acid groups in pulp fibers, it is necessary to ion exchange the pulps with a detectable monovalent ion.

In a novel procedure developed by Barzyk (5), the Ca^{++} ion concentration, and thus the carboxylic acid group concentration, was measured across the thickness of the cell wall. One

criticism of this technique is that relatively few (five) measurements could be taken across the thickness of the fiber wall. In addition, difficulty in aligning the electron beam causes uncertainty about the measurement near the surface of the pulp fiber.

ESCA ANALYSIS OF PAPER SURFACE

ESCA, or electron spectroscopy for chemical analysis, also called XPS, has been used to analyze the surface of chemically modified wood (50) or paper (24,51-61). Istone wrote an excellent overview of the use of ESCA in pulp and paper (62). In particular, ESCA has been used to determine differences in carboxylic acid content on the surface of the sheet (56, 57, 59). The technique uses x-ray photons to excite the electrons on the surface of pulp fibers. Electrons on the surface of the material are emitted when the energy of the incoming photons is greater than the binding energy of the electrons. The kinetic energy of the ejected photoelectrons is specific to the source atom, and is used to interpret the molecular makeup on the surface of the pulp fibers. The detector can distinguish between the photoelectron signals of most elements, and in the paper industry is commonly used for oxygen, carbon, calcium, etc. The carbon (C 1s) spectrum is divided into four peaks for the chemical states of the carbon atom. The peaks are labeled C1-C4. The C-H bond state corresponds to the C 1s-1 peak, C-O to the C 1s-2 peak, C=O to the C 1s-3 peak, and O-C=O to the C 1s-4 peak. The relative percent carboxylic acid groups for each sample of paper is determined from the size of these peaks. Prior to ESCA analysis, the sheets must be acetone extracted to remove wood extractives, which interfere with the ESCA measurement of carboxylic acid groups (61).

Stenius et al. (24) used ESCA to determine the surface properties of unbleached pine kraft pulp fibers prepared by digestion with different amounts of alkali. He was able to identify a relationship between carboxylic acid groups and alkyl carbon concentration with the precipitated lignin on the surface of the kraft pulp fiber.

Laine et al. (63) also examined the effects of alkali digestion conditions on the surface composition of unbleached pine kraft softwood fibers. Laine generated seven pulps with different kappa numbers by varying the effective alkali charge in the kraft cook. He then used the angular dependence of the total O/C ratio to identify a high concentration of alkyl carbons and carboxylic acid groups in a thin surface layer of precipitated lignin. His results were that the lignin concentration on the surface of the pulp decreased linearly with kappa number. Laine also found a strong enrichment of extractives on the pulp surface. The concentration of surface extractives increased with increasing effective alkali but was independent of digestion time. Laine then extracted the pulps with dichloromethane and measured the total extractives content with gas chromatography. In comparing total and surface extractives content, Laine concluded that extractives were precipitated onto the fiber surface during digestion and washing. Other researchers (119, 120) have confirmed the enrichment of extractives on the fiber surface.

ENZYMES

Enzymes are highly complex proteins that act as biological catalysts and are produced in all living things. They have the capacity to act as regulators, and can both stimulate and inhibit biological cycles. The International Commission on Enzymes was established in 1956. The commission created a systematic basis for enzyme nomenclature based on six recognized classes

of reactions. This system uses a four-digit numbering system to represent each class and subclass of enzyme (64).

Enzymes exhibit a high substrate and reaction specificity, which is a direct consequence of their complex three-dimensional structure. The enzyme-active site is normally a small fraction of the overall enzyme structure and is typically arranged in the shape of a cleft. The cleft structure is complementary to the structure of the substrate. This enables the enzyme to recognize only a few or sometimes one single substrate, and facilitates the selectivity of the enzyme (64).

The selectivity of the active site led to the "Lock and Key" model of reaction between the enzyme and substrate (65). Another model, called the "Induced Fit" model, allows for the ability of the enzyme to change its shape relative to the substrate. This allows for more flexibility in active site-substrate interactions because the protein can adjust to fit the substrate more precisely (64).

Enzyme activity varies greatly with respect to environment, pH, temperature, and the presence of organic solvents. The interaction of each of these solution conditions may affect the ability of the enzyme to react (64). The amount of enzyme added or used in a reaction is expressed as activity in katals. The katal is the amount of enzyme necessary to catalyze the conversion of one mole of substrate to its product in one second (64). Molar activity, or turnover number, k_{cat}, is expressed as the number of substrate molecules converted to products per enzyme per unit time, when the enzyme is saturated with substrate. The k_{cat} is a measure of the maximum catalytic activity of an enzyme.

ENZYMES IN PULP AND PAPER

Enzyme delignification studies have been performed for many years, generally using xylanases, mannanases, peroxidases, and laccases. Hemicellulases, including xylanase, were first studied because researchers believed that if the hemicellulose bonds could be broken, then lignin-hemicellulose fragments could be removed using alkaline extraction (66). Xylanase is believed to hydrolyze the precipitated xylan on the surface of the pulp fiber, and make the fiber more permeable. The increase in permeability is supposed to increase the number and size of the lignin and lignin-carbohydrate molecules that can be removed in subsequent chemical extractions (67).

Xylanases were one of the first enzymatic bio-bleaching treatments studied, and a large amount of research has been directed towards optimizing their use and understanding their reaction mechanism. However, xylanases and other hemicellulases lack the ability to degrade lignin, and, thus, there are limitations to their use in delignification of pulps (68).

White rot fungi produce laccase and peroxidase enzymes have the ability to degrade lignin. These enzymes are isolated from *Polyporus zonatus, Polyporus versicolor, Phellinus ignarius*, and other white rot fungi (69).

Direct treatment of pulp fibers with white rot fungi has had different degrees of success depending on the species of white rot fungi and the species of wood. After testing several white rot species, Paice et al. found that *Coriolus versicolor* produced the brightest pulp after a five-day treatment of hardwood pulp (70). The pulp brightness increased by 15 points, and the kappa number decreased from 11.6 to 7.9 with this treatment. Handsheet strength properties increased, while pulp viscosity dropped significantly. Further bleaching with a DED sequence produced a pulp with 82 ISO brightness, while a CEDED sequence produced an 88-brightness pulp (70).

OXIDOREDUCTASES

Enzyme isolation and characterization studies were performed to search for the agents involved in lignin degradation. The enzymes that are involved in lignin degradation are the aromatic alcohol dehydrogenases, phenol oxidases, and mono- and di-oxygenases: lignin peroxidase, manganese peroxidase, and laccase (67,69). These enzymes belong to the oxidoreductase class of enzymes, which are active in oxidation and reduction in the respiration and fermentation processes.

PEROXIDASE

Lignin and manganese (Mn) peroxidase delignification studies have been performed over the last ten years. This work has been done on ligno-cellulosic fibers, and lignin model compounds in the presence of H_2O_2 (71). Both lignin peroxidase and Mn peroxidase are composed of about 15% carbohydrates and contain iron atoms in their active site. The oxidation and reduction of these sites is believed to cause the degradation of lignin by numerous reactions. Both systems are dependent on the presence of H_2O_2, but excess peroxide will render the system inactive.

Early studies (69) on peroxidases reported that the enzyme diffuses into the fiber, but later it was found that the enzyme is too large to enter the fiber cell wall. This led to the introduction of mediator systems, where the enzyme interacts with a relatively small molecule, which can diffuse into the fiber (69).

Paice et al. proposed a Mn peroxidase mediator system for bleaching kraft pulps (68). In this system, the enzyme oxidizes Mn (II) in the presence of a chelant and H_2O_2, and the chelated Mn (III) diffuses into the pulp and oxidizes lignin. Mn peroxidase enzymes are dependent on the presence of Mn and are highly selective towards the degradation of lignin, with only very slight degradation of carbohydrates. With this system, the overall delignification is rather small, and the reaction time is in excess of five days. However, the selective ability of Mn peroxidase to demethylate the phenolic methoxyl groups appears to be high (68).

LACCASE

The laccase enzyme is produced by many types of fungi, and is classified as a blue oxidase in the oxidoreductase class of enzymes. The presence of copper in the enzyme accounts for the blue classification. Laccase is classified as EC 1.10.3.2 benzenediol:O_2 oxidoreductase, and is broken into subclasses depending on the organism which produces it, the molecular weight, and the number of central copper atoms (64).

Laccase usually contains four copper atoms (Cu II). The copper atoms are classified as of three types, depending on their differences in spectra (EPR), surrounding features, and accessibility to solvents. The spectrum for Copper 1 has a strong absorbance at 605 nm, while Copper 2 and 3 absorb at 280 nm. Copper 1 interacts with various solvents including water, can be removed by chelating agents, and displaced by mercury or cobalt. Copper 2 will interact with fluoride, and the surrounding protein undergoes conformational changes. Copper 3 can be removed from the enzyme with cyanide treatment, and subsequently re-embedded (72). Generally, there are one Copper 1, one Copper 2, and two Copper 3 atoms in laccase.

Transfer of electrons from one copper atom to another is believed to be responsible for the reduction of oxygen (see Figure 5). The primary acceptors of the electron in this oxidase are the amino acids cysteine and histidine, which transfer the electron to the Copper 1 atom. After Copper 1 has gained an electron, laccase can undergo a rapid reaction where oxygen is reduced to water (72). The active site of laccase has been studied in detail. It has been shown that the types 2 and 3 copper atoms function as a trinuclear copper complex with respect to oxygen and other ligands (73-77). Solomon suggested that the types 2 and 3 copper sites form a peroxide copper complex after binding oxygen to the active site (76). The proposed peroxide structure is shown in Figure 5.

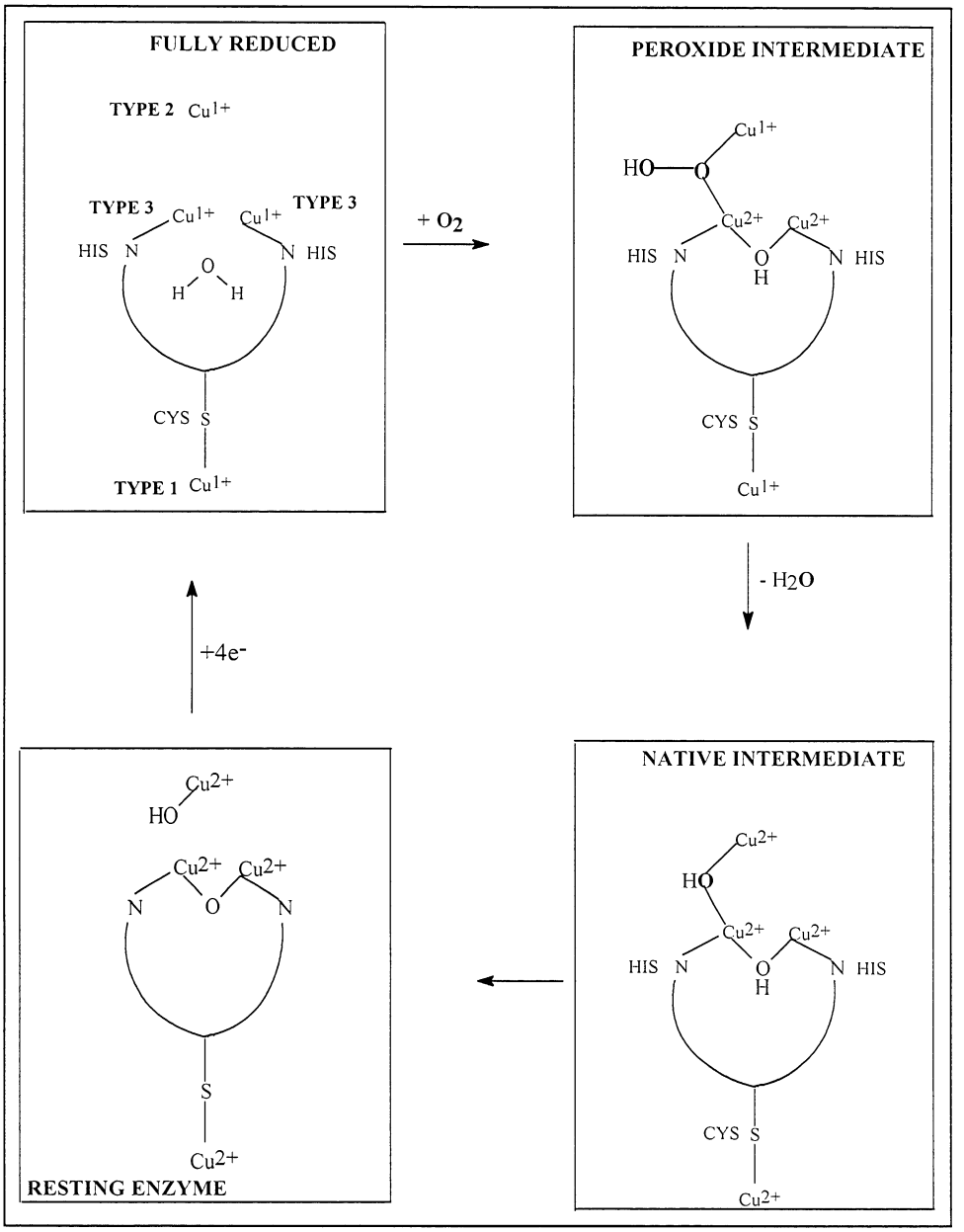

Figure 5: Proposed redox chemistry of laccase as presented by Solomon (77).

The peroxy-copper complex is further reduced by type 1 and type 2 copper to produce a molecule of water. The oxidative properties of these copper atoms are the key functional unit involved in oxidizing lignin, and in future bio-bleaching technologies.

Extensive study of laccase has been done for a variety of applications. The pulp and paper industry is interested in the ability of laccase to polymerize and depolymerize lignin while other

industries are interested in its use for organic synthesis (78,79). In the last fifteen years, numerous studies have been completed on the reaction of laccase with lignin model compounds. These studies have been carried out under optimum conditions, and the lignin model compounds contained bonding patterns that exist in native or industrial lignin. Laccase has been reported to degrade phenolic β-O-4 model compounds, β-1 linkages through Cα-Cβ cleavage, or by Cα oxidation, and to participate in ring cleavage (80). The proposed mechanism for ring cleavage of 4,6-di-t-butylguaiacol are shown in Figure 6. Researchers (80) showed that $^{18}O_2$ was incorporated into II, while $^{18}O_2$ from $H_2{}^{18}O_2$ was not incorporated into II. Based upon these labeling experiments, pathway A is favored over pathway B as the mechanism of degredation (80). This mechanism shows the production of carboxylic acid groups in lignin model compounds by laccase. Laccase has also been shown to catalyze polymerization reactions in lignin, and has been used to de-chlorinate bleach plant effluents and other compounds by polymerization of the aromatic materials (81).

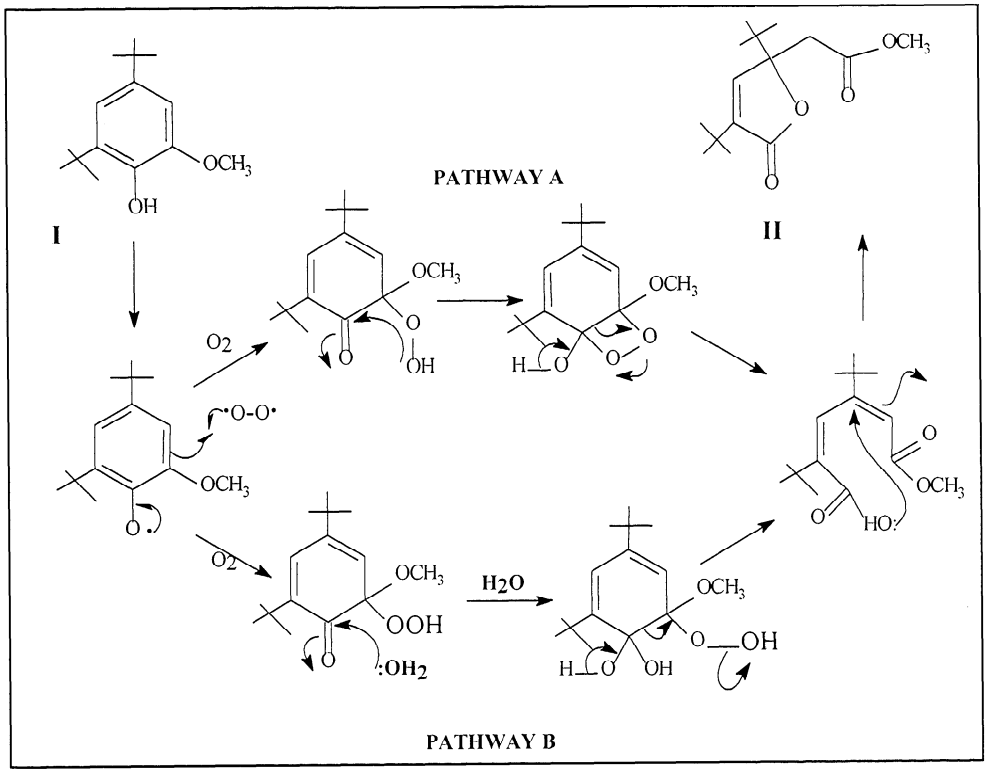

Figure 6: Possible mechanism for degradation of 4,6-di-t-butylguaiacol by laccase (as presented by Kawai) (80).

While these studies suggest that laccase alone could be an effective delignifying and brightening agent, laccase performs poorly with pulped fiber. This supports the view that the direct interaction between the enzyme and the lignin molecule is not the main mechanism of degradation, and that a mediator system is needed.

Laccase Mediator System

A laccase mediator system was introduced in 1990 with ABTS 2,2'-azinobis(3-ethylbenzthiazoline-6-sulphonate) as a mediator. Laccase and ABTS have been used successfully

to bleach hardwood kraft pulps (82). In that study, the system steadily reduced the kappa number from 32 down to 4 over five days, with only a small drop in viscosity from 24.2 to 21.9 mPa.

When laccase and ABTS are combined with oxygen at 100-400 kPa the delignification response was substantially improved. After alkaline extraction, 36.6 % delignification of softwood kraft pulp was achieved with only two hours of delignification (83). A control sample was used to determine that only 1.7 % of delignification could be attributed to the pressurized oxygen treatment. The laccase was recovered after reaction, and only 60% of its total activity was retained. Although ABTS is an efficient mediator for pulp delignification by laccase, a commercial application for this mediator does not seem feasible because ABTS was developed for analytical purposes, and its cost is too high for industrial pulp delignification (82).

The white rot fungi do not produce ABTS, and the exact function with laccase in lignin degradation is still unclear. Bourbonnais suggested that white rot fungi might produce effective mediators that can be used by laccase (83). This may be the actual mechanism of white rot fungi, but these mediators may be lost during isolation of the enzyme. This would explain their absence in isolated systems (69,96).

Call et al. presented a new laccase mediator system that has delignification properties comparable to conventional oxygen delignification systems (1,65,84). This system is effective for hardwoods and softwoods, before and after oxygen delignification. Delignification of over 60% of the residual lignin on oxygen delignified softwood kraft pulps can be achieved in several hours for a wide range of reaction conditions with little degradation of cellulose viscosity or tear and tensile strength. Tensile and tear strength measurements were made of the pulp from this study, and there was no reduction in either.

The proposed laccase-mediator delignification mechanism involves the reduction of oxygen, and the oxidation of a mediator, catalyzed by laccase, which selectively oxidizes the residual lignin. Figure 7 shows the proposed mechanism of reaction between oxygen, laccase and the mediator. Call's patent claims any mediator with the N-OH site, including hydroxybenzotriazole, but many different compounds might be used effectively as a mediator.

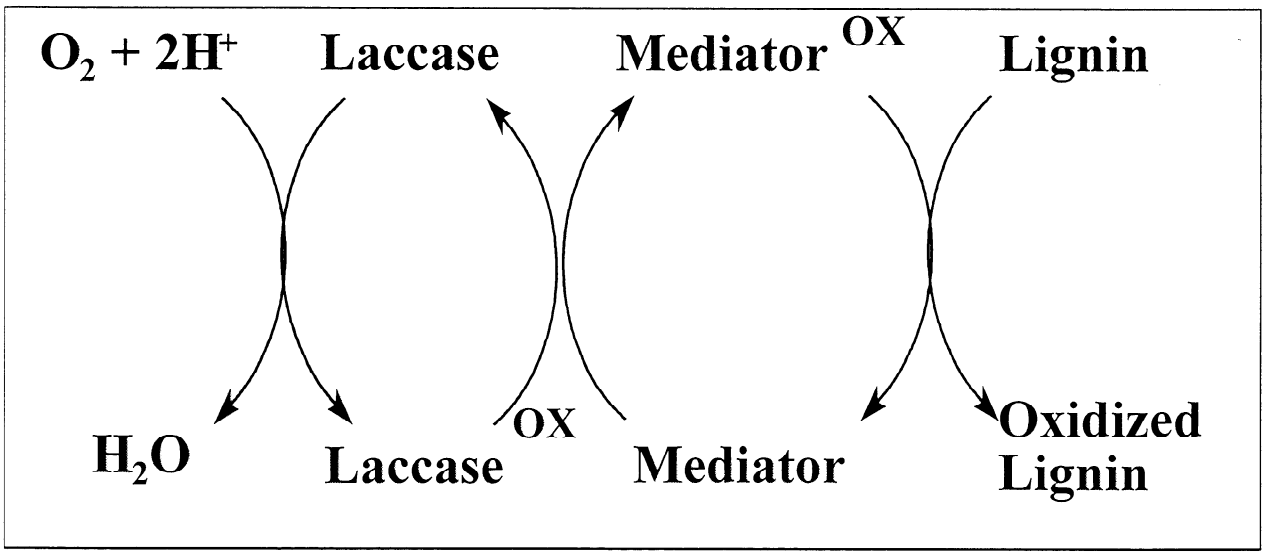

Figure 7: Interaction between the mediator and laccase (84).

Recent research has focused on the mechanism of reaction of laccase and mediators including ABTS and HBT (85-87), and on the discovery of new mediators (3,88,89). Amann reported two new mediators: N-OH-acetanilide (NHAA), and violuric acid (VIO). These new mediators are considered to be better candidates for mill scale because they have a lower nitrogen content, and are less expensive and more readily available than HBT. NHAA is also biodegradable. The mediators are shown below in Figure 8.

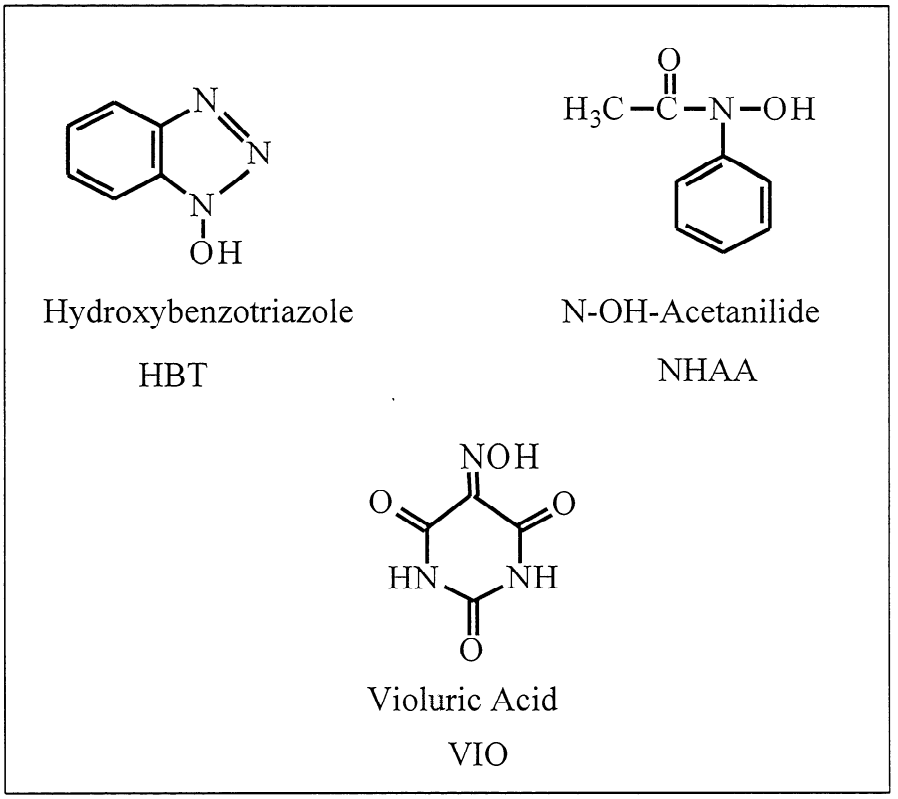

Figure 8: Laccase mediators hydroxybenzotriazole (84), N-hydroxyacetanilide, and violuric acid (88).

Research by Sealey revealed that the mediator HBT decomposes into benzotriazole (BT) in the presence of laccase (3). Conversion of HBT to BT during a laccase biobleaching stage reached a peak at 84% conversion after 24 hours (3). HBT also converts to BT in the presence of laccase alone, and the conversion is believed to be deleterious to the delignification reaction (3). HNAA and VIO also decompose into mediator conversion products in the presence of laccase (123). NHAA reacts with laccase to form N-phenylacetamide (NA) and also traces of NO-diacetyl-ohenylhydroxylamine (Diac-PHA) and notribenzene. VIO first forms an unstable imine, and then decmoposes to alloxan (123). The effects of these mediator conversion products on paper properties have not been studied.

OXYGEN DELIGNIFICATION

Oxygen delignification has emerged as an important technology worldwide as the search for economical environmentally friendly alternatives to the use of chlorine continues. The benefits of oxygen delignification are substantial, since the lignin content of pulp sent to the bleach plant is reduced by as much as 50% and total demand for chlorine dioxide and caustic is significantly reduced (16). Oxygen delignification is performed under alkaline conditions and under pressure (90). $MgSO_4$ is sometimes added to preserve viscosity. Usually a commercial oxygen delignification is carried out at alkaline pH with 3% NaOH on pulp, at medium consistency, 80-100 psi, 90-130°C for 20 to 60 minutes, with 0.01% $MgSO_4$ added to preserve viscosity.

Oxygen delignification has been shown to increase the number of carboxylic acid groups in lignin (91-94) and carbohydrates (5,94-95). Increases in the specific bond strength of pulp (16,91) have also been detected.

The introduction of carboxylic acid groups to pulp through laccase-mediator delignification raises the possibility that laccase-mediator treated pulps could have physical properties comparable to those of oxygen delignified pulps. The laccase-mediator delignification system could be an attractive alternative to oxygen delignification if the physical properties of laccase-mediator delignified pulp are similar to those of oxygen delignified pulps.

Both enzyme delignification and oxygen delignification are radical reactions. The laccase-mediator delignification mechanism is based upon a radical mediator species shown in Figure 7. In contrast, the oxygen delignification reaction involves several possible radical intermediates. The stepwise reduction of oxygen is shown below in Figure 9.

Step 1	$O_2 + e^- + H^+ \rightarrow HOO^\bullet$	$HOO^\bullet \rightarrow H^+ + O_2^{-\bullet}$	pKa = 4.8
Step 2	$HOO^\bullet + e^- + H^+ \rightarrow HOOH$	$HOOH \rightarrow H^+ {}^-OOH$	pKa = 11.8
Step 3	$HOOH + e^- + H^+ \rightarrow HOH + HO^\bullet$	$HO^\bullet \rightarrow H^+ O^{-\bullet}$	pKa = 11.9
Step 4	$HOH + e^- + H^+ + HO^\bullet \rightarrow 2\ HOH$		

Figure 9: Stepwise reduction of oxygen (122).

The product of the first step is the hydroperoxy radical ($HOO^\bullet$). However, this forms a weak acid, which remains uncombined under the alkaline conditions of oxygen bleaching. The hydroperoxy radical exists as the negatively charges superoxide anion ($O_2^{-\bullet}$) in alkaline bleaching conditions. The product of the second step is hydrogen peroxide. Both hydrogen peroxide and its anion the hydroperoxide anion (^-OOH) exist under oxygen bleaching conditions. The third step in the stepwise reduction of oxygen forms a hydroxyl radical ($HO^\bullet$). The hydroxyl exists in solution along with its anion ($O^{-\bullet}$). The hydroxyl radical can aquire another electron to form the hydroxide ion or water. Hydroxyl radicals are extremely reactive and indiscriminate, reacting with cellulose as well as lignin.

The complex oxidation processes that occur in oxygen bleaching involve radical chain reactions with a variety of organic species derived from both lignin and carbohydrates. Likely initiation, propagation and termination steps in the radical chain reaction mechanism of oxygen delignification are shown below (122) in Figure 10:

Initiation:	$RO^- + O_2 \rightarrow RO^{\bullet} + O_2^{-\bullet}$	Step 1
	$RH + O_2 \rightarrow R^{\bullet} + HO_2^{\bullet}$	Step 2
Propagation:	$R^{\bullet} + O_2 \rightarrow RO_2^{\bullet}$	Step 3
	$RO_2^{\bullet} + RH \rightarrow RO_2H + R^{\bullet}$	Step 4
Termination:	$RO^{\bullet} + R^{\bullet} \rightarrow ROR$	Step 5

Figure 10: Steps in the mechanism of oxygen bleaching (122).

In the first step, an electron is absorbed from a free phenolic hydroxyl group to form a phenoxy radical and a superoxide anion. Another possible initiation of the radical chain reaction is the removal of a hydrogen atom from an unionized phenolic group. This results in the formation of the corresponding organic radical ($R^{\bullet}$), and the hydroperoxy radical ($HO_2^{\bullet}$) in Step 2. Steps 3 and 4 are propagation steps, where an organic radical ($R^{\bullet}$) reacts with oxygen to form a peroxy radical ($RO_2^{\bullet}$), (Step 3). The peroxy radical ($RO_2^{\bullet}$) may in turn abstract a hydrogen atom to regenerate a new organic radical ($R^{\bullet}$), (Step 4). Coupling reactions such as that shown in Step 5 are responsible for the termination of the radical chain reaction.

Dissertation Objectives

The objective of this research is to examine the changes in fiber properties associated with the laccase mediator system. The introduction of carboxylic acid groups through enzyme treatment will also be studied. The hypothesis of this research is that the laccase-mediator systems can be used to introduce surface carboxylic acid groups to pulp fibers and improve the specific bond strength of these fibers. The objective will be accomplished in two research phases:

1. Determine the effects of laccase mediator delignification system on kraft fiber properties, and compare the results against similar pulps prepared with oxygen delignification.

2. Evaluate the laccase mediator delignification system, and variations of that system for carboxylic acid group topochemistry, and resulting effects on fiber properties for softwood kraft pulp with kappa 23. After identifying the enzyme delignification system that provides the greatest increase in surface carboxylic acid groups, evaluate the use of this system on kappa 50 pulp for changes in fiber physical properties.

Experimental Procedures

Materials and Supplies

Several pulps were used in this study (Table 1). Fully bleached Douglas-fir (*Pseudotsuga menziesii*) pulp was obtained from Weyerhaeuser Corporation. Kappa 23 and kappa 50 pulps were prepared from a 50-year-old loblolly pine (*Pinus taeda*) log from Union Camp in Savannah.

Table 1: List of pulps used in this study with kappa number and viscosity.

Pulp Type	Symbol	Kappa #	Pulp Viscosity (cP)
Fully Bleached Commercial Douglas-fir Pulp	FBP	-	20.9
Laboratory Prepared Loblolly Pine Pulp, Target Kappa 25	SKP23	23	33
Laboratory Prepared Loblolly Pine Pulp, Target Kappa 50	SKP50-1	51	41.2
Laboratory Prepared Loblolly Pine Pulp, Target Kappa 50	SKP50-2	51	41.2

All bulk chemicals (listed below) were purchased from VWR and were used as received: acetone, 1.00N NaOH solution, glacial acetic acid, 1.00N HCl solution, calcium carbonate, sodium chlorite.

All specialty chemicals (listed below) were purchased from Aldrich Chemicals company and were used as received: N-hydroxybenzotriazole (HBT), violuric acid (VIO).

The N-hydroxyacetanilide (NHAA) used in this study was synthesized in accordance with Oxley's method (97) by (3). The laccase in this study was Novo 504 provided by Novo

Nordisk. It was stored frozen, and thawed just before use. Only deionized water was used in this research.

Equipment

Pulps were treated in 100-gram increments in the "peg mixer." The reactor could be pressurized, and had temperature control and mixing capabilities. Reflectance measurements were done at 680 nm using an El-Repho 2000. These measurements were used to calculate scattering coefficient. Paper test equipment included the Instron tensile tester, and Scott bond tester. The HPLC used for carbohydrate analysis consisted of the following: The controller was a Waters 600E Multisolvent Delivery System. The Detector was a Waters 410 Differential Refractometer, and the column was a Bio-Rad Aminex HPX 87P with dimension 300x7.8mm. The conductivity meter was a E.C meter model 2052 by VWR.

Laccase Activity Measurements

The activity of laccase was measured by monitoring the rate of oxidation of syringaldazine. The change in A_{530nm} of 0.001 per minute per mL of enzyme solution in a 100 mM potassium phosphate buffer (2.2 mL) and 0.216 mM syringaldazine in methanol (0.3 mL) was set to one unit (U) of activity. This test was done at 23°C. (3)

NOVO activity measurements were performed with syringaldazine (19 μM) in a MES buffer (23 mM) at a pH of 5.5. The temperature was 30°C and the reaction time was 60 seconds. The activity was measured at an A_{530nm}. A laccase unit (LACU) was defined as: the amount of

enzyme that catalyzes the conversion of 1μmol of syringaldazine per minute. The MES buffer was prepared in the following way: 2.66 g of 2-(N-morpholine) ethane sulphonic acid was added to a 500-mL volumetric flask. One mL of 2 M NaOH was mixed with about 400 mL of distilled water and then added to the flask. Additional distilled water was added to bring the volume to 500 mL. The syringaldazine solution was prepared by adding 10.0 mg of syringaldazine to 50 mL ethanol (96%). Twenty-five mL of this solution was diluted with 25 mL of distilled water to make a 0.28 mM solution of syringaldazine. The MES solution (300μl) and the enzyme solution (100 μl) were mixed together for the activity measurement (98).

The type of laccase used in this research was SP 504 (recently commercially released as NS51002) provided by Novo Nordisk.. This laccase had an IPST activity of 584,000 U/mL, and a NOVO activity of 320 LACU/mL. The laccase solution has 3.2 mg protein per mL solution, and was isolated from the *Aspergillus fungus*.

Pulp Preparation

A 50-year-old loblolly pine log was obtained from Union Camp in Savannah. The log was debarked by hand, cut, and split into sections. The inner rings were discarded, and only the outer 20 years of growth were used in this study. Log sections were run through a four-foot Carthage chipper, and then screened in the shaker screen to remove oversize chip and fines. The chips used in this study were those collected on the ¾" and ½" screening trays. Chips were cooked in 1-kilogram (dry weight basis) batches. The reaction conditions are summarized in Table 2.

Table 2: Cooking conditions for SKP23 and SKP50-1 and –2. Cooks were done at Pioneer Canada Inc.

SKP23		**SKP50-1 and –2**	
Liquor to wood ratio	4 to 1	Liquor to wood ratio	4 to 1
Effective alkali	16%	Effective alkali	13%
Active alkali	18.8%		
Sulfidity	25%	Sulfidity	29%
H-factor	1450	H-factor	1017
Time from 0 to 170°	60 min	Time from 0 to 170°	60 min

The pulps were washed and screened (Iron Works screen with 8 cut slots), and the fines were removed by rinsing the pulp through a 200-mesh screen. Then the pulps were centrifuged and stored in the cold room until ready to use.

Care was taken from the start to minimize variation due to fines. Fines generated during pulping were removed by washing the pulp over a 200-mesh screen. In addition, all pulps were subjected to the same mechanical treatments of mixing and refining, and should have similar low fines content. In the laboratory, all pulps were washed and filtered in the same manner. With the removal of fines, and similar mechanical treatment, it is not likely that the limited variation in fines content contributed to changes in tested sheet properties.

This research is comprised of a complete and exhaustive investigation of the pulp and paper properties of laccase treated pulps. The pulp treatments are shown below in Tables 2-7.

Table 3: Samples included in Chapter 1, SKP 23 pulps treated with laccase and various mediators and then alkaline extracted.

Sample	Treatment
Untreated	Mechanical treatment in mixer, no chemicals added
E	Alkaline extraction
Lhbt-E	Laccase and HBT, followed by alkaline extraction
Lnhaa-E	Laccase and NHAA, followed by alkaline extraction
Lvio-E	Laccase and VIO, followed by alkaline extraction
O_2	Oxygen delignification

Table 4: Samples included in Chapter 2, SKP 23 pulps treated with laccase and various mediators.

Sample	Treatment
Untreated	Mechanical treatment in mixer, no chemicals added
Lhbt	Laccase and HBT
Lnhaa	Laccase and NHAA
Lvio	Laccase and VIO

Table 5: Samples included in Chapter 3, SKP 50-1 pulps treated with laccase and various mediators.

Sample	Treatment
Untreated	Mechanical treatment in mixer, no chemicals added
L	Laccase and no mediator
Lhbt	Laccase and HBT
Lhbt-E	Laccase and HBT, followed by alkaline extraction
Lnhaa	Laccase and NHAA
Lvio	Laccase and VIO

Table 6: Samples included in Chapter 4, SKP 50-2 pulps treated with single and repeat laccase VIO and oxygen delignification.

Sample	Treatment
Untreated	Mechanical treatment in mixer, no chemicals added
Lvio-E	Laccase VIO treatment, followed by alkaline extraction
Lvio-E-Lvio-E	Laccase VIO treatment, followed by alkaline extraction, and then repeated
O_2	Oxygen delignification
O_2-O_2	Repeated oxygen delignification

Table 7: Samples included in Chapter 5, acetone extracted SKP 50-2 pulps treated with laccase VIO.

Sample	Treatment
Untreated	Mechanical treatment in mixer, no chemicals added
A	Acetone extraction
A-Lvio	Acetone extraction, laccase and VIO
A-Lvio-E	Acetone extraction, laccase and VIO, followed by alkaline extraction

Fully Bleached Pulp

Fully bleached Douglas-fir (*Pseudotsuga menziesii*) wet lap pulp was obtained from a Weyerhaeuser mill and used in this study. The pulp was at about 40% consistency, and kept cold until ready for use. The fully bleached pulp (FBP) was subjected to the following treatments:

Table 8: Samples included in Chapter 6, FBP treated with laccase and various mediators.

Sample Designation	Treatment
Untreated	Mechanical treatment in mixer, no chemicals added
E	Alkaline Extraction
O_2	Oxygen Delignification
Lhbt-E	Laccase HBT treatment, followed by alkaline extraction
Lnhaa-E	Laccase NHAA treatment, followed by alkaline extraction
Lvio-E	Laccase VIO treatment, followed by alkaline extraction
Lvio-E-Lvio-E	Laccase VIO treatment, followed by alkaline extraction, and then repeated

Enzyme and oxygen treatment

Treatments were done in 100grams, oven dried batches in the "peg mixer." This stainless steel reactor can be pressurized, temperature controlled, and stirred. The procedure for an enzyme treatment was:

1. Pre-heat pressure reactor and pulp to 45°C at 10% consistency.
2. Add mediator at 2% on pulp for HBT, and molar equivalents of this amount for VIO and NHAA. Mix thoroughly for two minutes.
3. Adjust pH to 4.5 with glacial acetic acid if necessary.
4. Add 1.75 X 10^6 U of batch SP 504 Novo Nordic Laccase Enzyme (30 mL) per 100 grams of pulp. Stir thoroughly for two minutes.
5. Close pressure reactor and pressurize reactor with oxygen (80 psi).
6. Stir 4 hours at 45°C at constant 80 psi oxygen pressure.
7. Remove and wash pulp.

When the enzyme treatment was to be followed by alkaline extraction, the extraction stage was preformed at 10% consistency with 2% NaOH for 1 hour at 70°C in a water bath. The pulp was then thoroughly washed with DI water.

Oxygen delignification treatments were done in the "peg mixer." The O_2 delignification procedure was:

1. Pre-heat pressure reactor and pulp to 95°C at 10% consistency.
2. Add 2% NaOH on pulp, close pressure reactor, and continue heating to 100°C.
3. Pressurize the reactor with oxygen (60 psi).
4. Stir 1 hour at constant 60 psi oxygen pressure.
5. Remove and thoroughly wash pulp with DI water.

Control pulps were treated in the peg mixer without the addition of chemicals to ensure that all pulps had similar mechanical work (curl) history. This procedure was:

1. Pre-heat pressure reactor and pulp to 45°C at 10% consistency.
2. Adjust pH to 4.5 with glacial acetic acid if necessary.
3. Stir 4 hours.
4. Remove and thoroughly wash pulp with DI water.

Acetone extraction

Several SKP51-2 pulps were acetone extracted prior to enzyme treatment. This allowed the effects of wood extractives to be determined. The extraction was carried out in two large soxhlets. Fresh acetone was used, and the slurries were soxhlet extracted for 24 hours. The pulp was then removed from the soxhlets and soaked in water overnight. In the morning the pulp was filtered and rinsed thoroughly with DI water.

Acetone extraction was also done on handsheet samples prior to ESCA analysis. These samples were placed in smaller soxhlets, and allowed to reflux for 12 hours. The samples were then removed from the soxhlets and allowed to air dry thoroughly.

Pulp Classification

After an enzyme or an oxygen treatment, the pulps were converted into their calcium form for testing. This ion conversion was accomplished by the following method: Pulps were first treated at room temperature with sulfuric acid to pH 2.75 at about 1% consistency. The pulp was mixed for ½ hour to acidify the acid groups. The pulps were then rinsed thoroughly with distilled water. Saturated calcium hydroxide solution was added until a pH of 10 was reached, and the pulp was stirred for ½ hour, to allow the calcium ions to exchange with the acid groups. The pulps were washed again with deionized water and stored cold. All pulps were converted to the calcium form prior to testing. Pulp tests included kappa number, modified Klason lignin, viscosity, acid group content by titration and carbohydrate analysis.

Kappa Number

Kappa number and Klason lignin are measures of the amount of oxidizable lignin material in the pulp. Kappa numbers were measured in accordance with TAPPI useful method UM-246 "Micro Kappa Number." This procedure requires only 1-3 grams of pulp, and was used due to the limited sample size. The standard deviation for kappa number is $\pm$ 0.3.

Klason Lignin

The Klason lignin procedure was modified from the TAPPI standard procedure to accommodate smaller sample sizes (115,116). This procedure required about two grams of pulp for SKP23 and one gram of pulp for SKP51-1 and -2. Klason lignin was measured in accordance with the following procedure: the appropriate amount of pulp was weighed out, and placed in an

autoclave bottle; 24 mL 72% H_2SO_4 was then added and the solution was mixed with a glass rod for 5 minutes. The autoclave bottle was placed in a water bath at 30°C for 1 hour, and mixed occasionally with the glass rod. The mixture was then diluted to 4% acid with distilled water, and autoclaved at 120°C for 1 hour. The bottle was allowed to cool, and its contents filtered through a tared medium fritted glass crucible. The acid insoluble lignin material remaining in the crucible was washed with distilled water. The crucible was then placed in a 105°C oven for 24 hours and then weighed. The lignin content was calculated with a standard deviation of $\pm$ 0.02%.

Viscosity

Viscosity values were determined in accordance with TAPPI method T-230 om-89 "Viscosity of Pulp (capillary viscometer method)." Consistency was determined on air-dried pulp, and the pulp was weighed out to $\pm$ 0.0005 g. All pulp solutions were monitored for insoluble material. Data was not reported for samples where insoluble material was found. Viscosity measurements were reported in centipoise, and have a standard deviation of $\pm$ 0.6cp.

All viscosity measurements for SKP 50-1 and 50-2 were done on pulp following acid chlorite treatment. The acid chlorite treatment was performed as follows: Place one gram of sodium acetate, 3 g of sodium chlorite, and 2 mL of acetic acid in a 50-mL volumetric flask. Fill to mark with DI water and mix. Pour this mixture over one oven-dried gram of pulp in a 125-mL Erlenmeyer flask with screw top. Swirl the flask, and allow it to stand overnight. The following morning, the mixture was filtered through a course sintered glass funnel equiped with filter paper. The sample was then washed thoroughly with distilled water.

Acid group titration

Bulk acid group content was measured by titration. The acid group titration procedure was modified from TAPPI standard T237 om-9. The moisture content of the pulp was first determined, and 1.5 od g of pulp was weighed out into a 400-mL beaker; after which 300 mL of a 0.1 M HCl solution was added to the beaker, and the solution was stirred for one hour. The pulp was then filtered using a vacuum flask and coarse fritted glass filter funnel. The pulp was washed with 400 mL deionized water, and transferred back to the 400-mL beaker. Next 250 mL 0.001 M NaCl was added to the beaker, followed by 1.5 mL of a standardized 0.1 M HCl solution. The solution was stirred while nitrogen gas was bubbled through it. Initial conductivity was measured and recorded. The solution was titrated with 0.25 mL aliquots of 0.05 M NaOH and the conductivity measured after each 0.25 mL addition. The titration and conductivity measurement was stopped after 10 mL of 0.05 M NaOH was added. The conductivity was plotted vs. mL of NaOH, producing a parabolic shaped curve. Lines were drawn through the linear portions of the graph, and the flat or horizontal portion of the curve. Point A is defined as the intersection between the downslope portion of the graph and the horizontal line. Point B is defined as the intersection of the horizontal line with the line drawn through the upslope portion of the graph. The carboxylic acid concentration was calculated as:

$$(\text{COOH meq/g}) = ((B-A)*N_{NaOH})/W_{od}$$

Where A is the interception of the downslope with the horizontal line, B is the intersection of the upslope with the horizontal line, and C is the mL NaOH. N_{NaOH} is the normality of NaOH, and W_{od} is the oven-dry weight of wood. The standard deviation of this procedure is $\pm$ 0.006meq/g.

Nitrogen analysis

Nitrogen analysis was done at Huffman Labs in Golden Colorado. The samples were dried at 100°C for two hours prior to analysis. Results were reported on a dried sample basis.

Carbohydrate analysis

Carbohydrate analysis involves the separation and quantification of wood sugars using HPLC analysis (standard deviation is ± 0.2% xylan content and ± 0.4% for cellulose). The procedure is as follows:

1. Mill the pulp with a Wiley Mill to pass through a 40-mesh screen.
2. Weigh out 0.350 grams of air-dry pulp, to the nearest 0.0001 g, into a digestion vessel.
3. Add 3 mL of 72% H_2SO_4 to the pulp and mash it.
4. Place mixture in a 30°C bath for one hour, mashing the pulp/acid mixture every ten minutes. This time may be extended if clumps of pulp are visible.
5. Dilute the mixture with 50 mL nanopure water, using a rubber policeman to clean the stirring rod.
6. Cap the vessel and autoclave for one hour using liquids cycle. Cool the vessel in ice water.
7. Filter solution into a 250 mL graduated cylinder, and add nanopure water to 150 mL.
8. Pour solution into beaker to mix.
9. Remove 20 mL aliquot and add 2 mL Erythritol Internal Standard (IS) solution.
10. Neutralize to pH 5.3 with $Ba(OH)_2$ using autotitrator.
11. Transfer neutralized solution to a 50-mL centrifuge tube, and centrifuge for 10 min at 5 x 1000.
12. Filter through #2 filter paper into a 100-mL round bottom and concentrate to approximately 2 mL using rotovap. Filter through a 0.2 μm PVDF filter.
13. Equilibrate column for 30 minutes at 0.6 mL/min water at 85°C. Inject 100 μl aliquot of the filtered sample solution.

The glucose concentration in this technique has a poor level of accuracy, but the xylose, galactose, arabinose and mannose are resolved with a high level of accuracy (117,118). Due to the limitation of this carbohydrate analysis, comparisons will only be made between xylose, galactose, arabinose and mannose.

HANDSHEET FORMATION AND CHARACTERIZATION

In this study, pulps were refined to the equivalent of 1500 revolutions in a PFI mill. Pulps in the first three chapters were refined at Weyerhaeuser to 1500 revs. Pulps in the last three chapters were refined at IPST to 500 revs. (500 revolutions at IPST is equivalent to 1500 revs on a standardized pulp.) This low level of refining was done to remove curl and kinks, and should not generate much change in fines content.

After refining in a PFI mill, the pulp was disintegrated for 15,000 revolutions in a laboratory disintegrator. TAPPI handsheets were formed in accordance with TAPPI method T205 om-88. Six handsheets were made at each wet pressing pressure for each pulp sample. Wet pressing pressures were 5, 50, 95, and 500 psi. A standard laboratory press was used for the 5, 50 and 95 psi sheets, and a carver press was used for the 500 psi sheets. The carver press sheets were pressed to 15,300 total pounds, which corresponded to 500 lbs/inch^2. All sheets were pressed 5 minutes on one side, and 2 minutes on the other, in accordance with TAPPI standards. Distilled water was used in the production of all handsheets. Handsheets were tested for tensile, zero span tensile, Scott bond, and scattering coefficient.

Tensile breaking length was measured in accordance with TAPPI T220 om-88. Basis weight and hard caliper were measured first. For the tensile test, strips were cut 15 mm wide and 4 inches long. Tensile breaking length was calculated from the tensile measurement. Elastic modulus is a product of the tensile test measurement. Zero span breaking length of pulp was measured in accordance with TAPPI Classical Method T231 cm-85 "Dry Zero Span Breaking Length of Pulp." The standard deviation of the zero span tensile test measurement is $\pm$ 9.5Nm/g.

Scott bond strength was measured using a Scott bond tester. Two strips of paper, one inch wide by five inches long, were cut for each test specimen. Each strip was used to perform five Scott bond tests, and only one strip was used, unless the test needed to be repeated because the tape pulled up. The samples were fixed between two metal plates with tape. A pendulum was used to strike the top plate, separating the paper. The height of the pendulum swing was measured by the instrument, and used to calculate the Scott bond strength.

Scattering coefficient was calculated from reflectance measurements recorded at 680 nm on the El-Repho 2000. Three squares, 41 mm to a side, were cut for each sample, and placed in the optics lab for 24 hours to condition. Each square was weighed to establish the exact basis weight of the sample, and reflectance of the sample was then measured at 680 nm over a black void and a white tile. These measurements were used to calculate the scattering coefficient of the samples. Parameters used on the El-Repho 2000 were: 33-mm diaphragm size, 32-mm spot size, and 69.5 UV% with the UV filter out.

To test that 680 nm was adequate to avoid absorption interference, the scattering coefficient was measured on SKP 50-1 pulp samples at 950 nm. This measurement was done at Paprican. These measurements were compared to those at 680 nm. There were only slight differences between the data at 680 and at 950 nm, and all measurements of SKP 50-2 were recorded at 680 nm at IPST.

Brightness measurements were made on handsheets according to TAPPI standard T452, and the standard deviation was $\pm$ 0.47.

ESCA analysis

Electron Spectroscopy for Chemical Analysis (ESCA) measurements were done in Finland at the Helsinki University of Technology analysis laboratory. The instrument used was an AXIS 165 (by KRATOS Analytical). Prior to ESCA analysis, handsheet samples were acetone extracted in soxhlets for 12 hours to remove extractives. This was critical to the ESCA measurement of extractives as extractives interfere with the ESCA measurement. Five measurements were done for the following samples:

SKP 23: Untreated, Untreated-E, Lvio-E, O_2, Lhbt, Lnhaa, and Lvio.
SKP 50-1: Untreated
SKP 50-2: Untreated, Lvio-E, Lvio-E-Lvio-E, O_2, O_2-O_2, A, A-Lvio, A-Lvio-E.
FBP: Untreated, E, O_2, Lhbt-E, Lnhaa-E, Lvio-E, and Lvio-E-Lvio-E.

Three measurements were done on the following samples:

SKP23: Lhbt-E, Lnhaa-E.
SKP50-1: L, Lhbt, Lhbt-E, Lnhaa, and Lvio.
SKP 50-2: Untreated, Lvio-E, Lvio-E-Lvio-E, O_2, O_2-O_2, A, A-Lvio, A-Lvio-E.
FBP: Untreated, E, O_2, Lhbt-E, Lnhaa-E, Lvio-E, and Lvio-E-Lvio-E.

Initial ESCA measurements were made on paper samples known to have different surface acid group contents. These samples were provided by Dr. Barzyk. The results of ESCA measurements agreed with Dr. Barzyk's research, and are shown in Table 9. Five measurements were recorded for each sample, and have standard deviations of $\pm$ 0.08%. This establishes ESCA as a viable means of measuring surface carboxylic acid content.

Table 9: ESCA measurements made on Dr. Barzyk's handsheet samples, showing increase in C 1s 4 orbital (acid group bound) carbons on the surface treated sheet.

Sample	ESCA measurement of % C 1s-4 orbital bonded atoms
Uniform, 6-200-1	0.548
Blank, 7-200-3	0.542
Surface, 5-200-3	0.913

ESCA Procedure

Each measurement included two spectra: wide and high-resolution calcium and oxygen spectra. Prior to measurement handsheet samples were acetone extracted to remove wood extractives which interfere with the test measurement. The experimental procedure was as follows:

1. Two small pieces were cut from the middle of the sheet and attached to the sample holder with a metal clip (no adhesives were used in order to minimize contamination).
2. The samples were evacuated in the pre-chamber overnight for removal (degassing) of water and to ensure that all samples were measured under standard conditions.
3. three or five measurements were made at different points on the sample. The analysis volume is less than 1 square millimeter in diameter and between 2 - 10 nm in depth (the latter depends on the kinetic energy of the electron signal studied).
4. As the samples were insulating, they were neutralized with slow electrons during the measurement. This caused a shift of ca. -2.5 eV in binding energies throughout the spectra. The high-resolution spectra were charge-corrected using the tabulated value of 285.0 eV for the C 1s (1s orbital) components (C-C bond). However, the concentration tables are done on non-shifted data.

Table 10: Run Parameters for wide and high resolution spectrum.

Wide Spectra:	High resolution spectra:
Irradiation: Monochromated Al Ka (12 kV, 8 mA)	Irradiation: Monochromated Al Ka (12 kV, 8 mA)
Binding energy range: 0-1100 eV	Elemental regions
	C 1s: 278-298 eV
	O 1s: 523-543 eV
	Ca 2p: 337- 360 eV (two samples only)
Analyzer Pass Energy: 80 eV	Analyzer Pass Energy: 20 eV
Step: 1 eV	Step: 0.1
Lens: hybrid	Lens: hybrid
Aperture: slot	Aperture: slot

Peak fitting parameters were based on the labs internal database/experience on extracted cellulose samples. Component fitting was done with four Gaussian peaks, all having the same peak width at half maximum; distance between peaks (relating to chemical shifts of known species) were also held constant during the iterations. The distance between C-C and C-O carbons was modified slightly for some samples (± 0.05 eV) in order to make a smooth error function line: in that case all the measurements for that sample were shifted similarly. None of the other fitting parameters needed modification, as fitting produced smooth error lines.

Note that in the concentration tables all binding energies are some 2.5 eV lower, due to charge neutralization, which shifts the eV scale. Component numbering may vary from fit to fit, but the peak position indicates which component is in question. As a comparison, tabulated values and Laine tabulations are shown in Table 11. ESCA standard deviation is $\pm$0.08%.

Table 11: Measured binding energies and Laine tabulations for carbon bonds (63).

Orbital	binding energy	Laine tabulations	Chemical state
C 1s-1:	true value 285 eV		C-C, C=C, etc.
C 1s-2:	+ 1.8 ± 0.05 eV	+ 1.7 ± 0.2 eV	C-O
C 1s-3:	+ 3.2 ± 0.05 eV	+ 3.1 ± 0.3 eV	C=O or O-C-O
C 1s-4:	+ 4.3 ± 0.05 eV	+ 4.4 ± 0.3 eV	Carboxyl groups

An example of the ESCA equipment is shown in Figure 11. The diagram shows the x-ray source, lens, analyzer and detector.

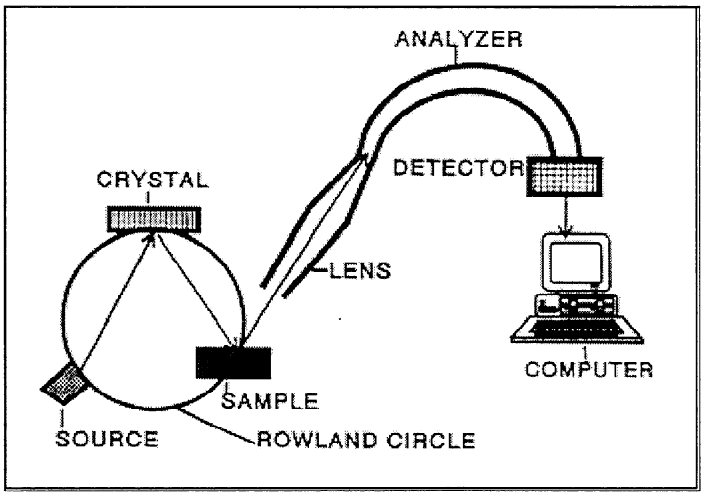

Figure 11: Diagram of a modern x-ray photoelectron spectrometer employing a spherical analyzer (SCA) and a monochromatic x-ray source (as published in 62).

An example of a typical carbon spectra is shown in Figure 12. The relative shapes of the carbon orbital peaks are shown.

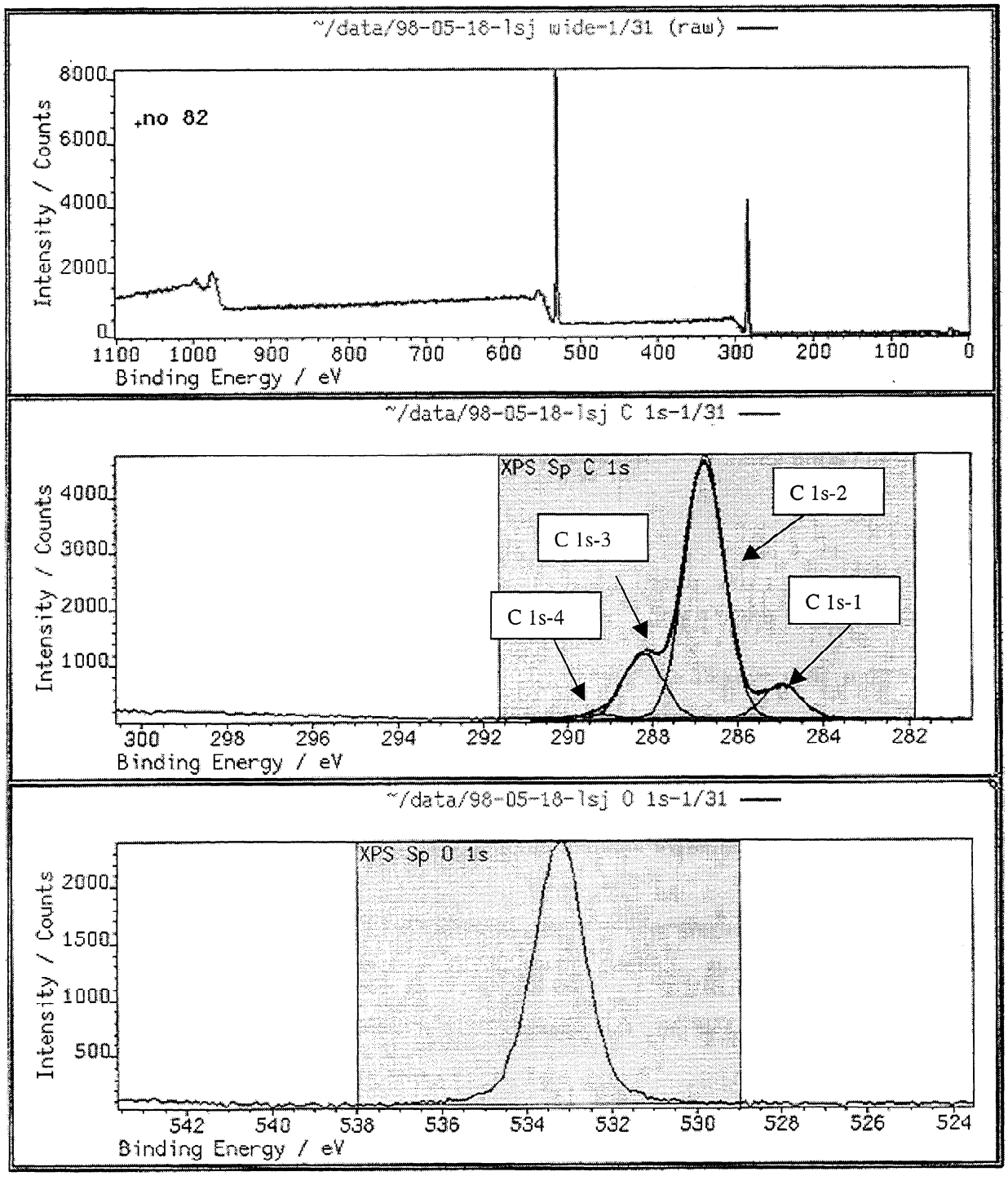

Figure 12: Typical spectra for ESCA analysis. The top spectrum is the wide spectrum analysis showing the carbon and oxygen peaks. The middle spectrum is the carbon analysis showing the separate peaks for each orbital. The bottom spectrum is the oxygen peak.

Statistics

Statistical analysis of physical properties was done using analysis of covariance. Where data is shown graphically, linear regression was done to compare each line with the appropriate control. This test was done to detect statistical differences in both intercept and slope. Probabilities are located in the caption below each chart. Raw data were averaged prior to statistical analysis. The regression lines generated in the analysis of covariance had a standard error equal to the standard deviation of the averaged data points. Raw data and standard deviations are located in the appendix.

Standard deviations were calculated from pooled variances for each of the following tests: kappa number, viscosity, ESCA, bulk acid group content, acid insoluble lignin, brightness, and zero span tensile data.

X-ray Analysis of Fibril Angle

X-ray work was done using a General Electric XRD-7 diffractometer. The procedure followed that described by El-OSTA (121). Six samples (3 earlywood, 3 latewood sections) were randomly measured using the strong 002 equatorial reflection pattern. The beam was introduced to the tangential face of each sample while each spun 360°. Two peaks were produced from each sample and the Micro Fibril Angle (MFA) produced is the average derived from the two peaks. The X-ray analysis is reproducible to ± 0.8° using standard samples. However, the large standard deviation obtained from the analysis is primarily the result of the variability found within the sample population.

X-ray Specs:

Earlywood and latewood samples were calibrated independently of one another using a light microscope and hand section of compression-wood-free loblolly pine; base width of the diffraction patterns correlated to MFA (121).

Equations:

Latewood MFA = Peak Width (inches) * 15°/inch – 15.75°
Earlywood MFA = Peak Width (inches) * 13.65°/inch – 9.75°

The average fibril angle for the laboratory pulp used in this study was 16°, the micro fibril angle measurement was also 16°, with a standard deviation of 6°. The raw measurements are shown below in Table 12 and Table 13.

Table 12: Earlywood fibril angle measurements made using x-ray analysis.

Earlywood Samples	Peak Width 1	Peak Width 2	Mean Fibril Angle (early)
1	2.07	2.0705	15.3
2	2.5985	2.7375	24.3
3	2.385	2.5265	21.1
		Average	20.2

Table 13: Latewood fibril angle measurements made using x-ray analysis.

Latewood Samples	Peak Width 1	Peak Width 2	Mean Fibril Angle (late)
1	1.794	2.07	16.6
2	1.281	1.26	7.6
3	1.702	1.64	13.1
		Average	12.4

RESULTS AND DISCUSSION

PREFACE

The results and discussion section is divided into six chapters. Chapter 1 covers the pulp and paper physical properties of kappa 23 laccase-mediator delignified pulp, when oxygen delignification was used as a standard for comparison. The control pulp in this case was the alkaline extracted pulp, since all of the treatments included an alkaline extraction stage.

In Chapter 2 the pulp and paper physical properties of laccase-mediator treated kappa 23 pulp without an alkaline extraction were investigated. Omitting the alkaline extraction stage allows determination of changes in paper physical properties due to the presence of the oxidized lignin material or extractive material in the pulp. These pulps are compared to an untreated control pulp.

In Chapter 3 the possibility of using laccase-mediator chemicals to modify high kappa (kappa 50) pulp fibers was investigated. The fibers were treated with laccase-mediator systems, and their physical properties investigated.

In Chapter 4 the combination laccase-violuric acid and alkaline extraction was used as a single and double treatment for kappa 50 pulp. Laccase-violuric acid was chosen because it is the best combination for delignification. The results were compared with both single and double oxygen delignification. The chemistry involved in both oxygen delignification and laccase-mediator delignification was used to explain the results.

The role of wood extractives on the laccase-violuric acid delignification of high kappa pulps was investigated in Chapter 5. Kappa 50 pulp was acetone extracted to remove wood

extractives, and then treated with laccase-violuric acid delignification systems. The results from this chapter are compared with those in Chapter 3.

In Chapter 6 the possibility of modifying fully bleached pulps with laccase-mediator systems was investigated. The results were compared with oxygen delignification. The control pulp is an alkaline extracted pulp, since all of the treatments included an alkaline extraction stage.

CHAPTER 1

COMPARISON OF PHYSICAL PROPERTIES OF LACCASE MEDIATOR DELIGNIFIED KAPPA 23 PULP FOLLOWING ALKALINE EXTRACTION

INTRODUCTION

Recent improvements to the laccase mediator system have yielded new bleaching treatments that can achieve greater than 50% delignification (1). These advances have occurred due to the development of new mediators that are more effective and environmentally friendly (2). These improvements bring the laccase mediator system closer to mill application. Before this technology can be effectively deployed, several challenges remain, including the need to establish the effects of laccase mediator delignification on paper physical properties. With few exceptions (99,100) the effects of laccase mediator delignification on fiber and paper properties have been largely ignored.

Viikari et al. (99) studied the effects of laccase and laccase-HBT treatment on mechanical pulp fibers. She found that laccase-HBT induced the polymerization of lignin. This caused an increase in the strength of the handsheets made from mechanical pulp fibers. She did not investigate the effects of laccase-HBT on handsheet properties of chemical pulps.

Hassingbow et al. (100) measured the effects of laccase with no added mediator on bonding in medium density fiberboard. By extracting mechanical pulp fibers with a variety of solvents, including water and acetone, he was able to examine the effects of these components on oxygen consumption during laccase treatment. He found that these water-soluble components enhanced oxygen consumption in laccase treatment, which suggested that these components

might include a biological mediator. He also found a slight increase in modulus of elasticity and modulus of rupture for air-laid and hot-pressed mechanical pulp fiberboards.

Research suggests that the laccase mediator system introduces carboxylic acid groups to pulp fibers (3), but it is not clear whether the acid groups are located on the lignin or hemicellulose pulp fractions.

Laccase alone is too large to fit inside the pores of a kraft fiber (69), but may have the ability to react with the surface of the pulp fiber. The modification of fiber surface properties is of particular interest. Acid groups on the surface of pulp fibers have been shown to improve specific bond strength (5) and tensile strength (101).

This chapter compares the pulp and paper physical properties of laccase mediator delignified kappa 23 loblolly pine kraft pulp fibers with those of oxygen delignified pulp fibers. The pulps in this chapter are compared with an alkaline-extracted, but otherwise untreated, pulp. ESCA measurement of surface acid content has been established as a reliable means of comparing surface carboxylic acid concentrations. ESCA, together with bulk acid group measurement through titration, allows the determination of changes in surface and total pulp acid group content.

The measurement of surface and bulk acid content, together with paper physical properties, allowed the study both the laccase mediator system and the changes in paper physical properties due to acid groups.

RESULTS AND DISCUSSION

PULP PROPERTIES

Table 14 shows the pulp properties for both laccase mediator delignified kappa 23 (SKP23) pulp and oxygen delignified kappa 23 (SKP23). The table includes measurements of kappa number, viscosity, ESCA analysis, bulk acid group content, brightness, and zero span tensile. The initial kappa number of the pulp was 21.9 after alkaline extraction. Of the mediators studied, VIO was far more effective at delignification, reducing the kappa number from 21.9 to 14.2 (a 35% drop in kappa). Laccase-NHAA treatment, followed by alkaline extraction (Lnhaa-E) was less effective at decreasing kappa number, with only a 2.5 kappa drop. Laccase-HBT treatment followed by alkaline extraction (Lhbt-E) only decreased the kappa number by 2.8 to 19.1 kappa, a decrease of only 13%. The oxygen delignification stage caused a 4.9 kappa drop.

Viscosity did not decrease substantially for Lnhaa-E or Lhbt-E. There was only a slight decrease in viscosity for Lvio-E delignification (from 32 to 30cp), but a much larger decrease for the oxygen delignified pulp (from 32 to 22cp). This indicated that the laccase-mediator delignification chemistry is selective with respect to lignin and does not degrade the cellulose fraction of the pulp.

ESCA measurement of surface acid groups is given in % of total atoms on the surface. The surface acid content increased slightly with alkaline extraction. This result is not statistically significant, but it is in agreement with previously published research (94,95). Lvio-E caused a statistically significant increase in surface acid content. From our current understanding of theory, this should result in an increase in bond strength. Oxygen delignification did not cause a significant increase in surface acid content over the alkaline extraction stage.

Bulk acid group content increased for the alkaline-extracted pulp, again in agreement with previously published work (94,95). None of the delignification treatments increased the bulk acid content further, although Lnhaa-E and Lhbt-E caused a slight decrease.

Acid insoluble lignin results agreed closely with the kappa number results. Of the three mediators, Lvio-E was the most effective at removing lignin. All of the mediators caused a decrease in brightness, particularly Lnhaa-E, while oxygen caused an increase in brightness. Zero span tensile strength did not change for any of the treatments.

Table 14: Pulp properties of kappa 23 (SKP23) pulp following laccase-mediator delignification and alkaline extraction.

Sample	Kappa #	Viscosity (mPa/s)	ESCA COOH- (%) [1]	Bulk Acid Content (meq/g) [2]	Acid Insoluble Lignin (%)	Bright-ness (ISO)	Zero Span Tensile (Nm/g)
Standard deviation	±0.3	±0.6	±0.080	±0.006	±0.02	±0.5	±9.5
Untreated	23.0	33.1	0.799	0.063	4.40	21.1	129.7
Untreated-E	21.9	32.0	0.881	0.072	3.40	21.9	126.7
Lhbt-E	19.1	32.1	1.011	0.063	3.20	19.8	127.3
Lnhaa-E	18.8	32.2	0.932	0.060	3.40	16.1	128.1
Lvio-E	14.2	30.0	1.064	0.070	2.40	19.5	129.2
O2	17.0	22.3	0.915	0.070	3.00	23.8	125.7

[1] Surface carboxylic acid content in % total atoms responding.
[2] Bulk acid group content in meq/g of oven dried pulp.

Carbohydrate analysis of these pulps is shown in Table 15. As mentioned in the experimental, there is a limitation with the technique. There is a trend toward the removal of xylan for Lhbt-E and Lvio-E, but no statistically significant difference in the data.

Table 15: Carbohydrate analysis of kappa 23 (SKP23) alkaline-extracted pulps. (Standard deviation ± 0.5 for xylan and mannan)

	Glucan %	Xylan %	Galactan %	Arabinan %	Mannan %
Untreated	71.1	5.5	1.0	0.4	6.5
Untreated-E	75.2	5.4	nd	0.3	6.6
Lhbt-E	78.8	4.6	nd	0.2	6.3
Lnhaa-E	78.3	6.1	nd	0.6	7.2
Lvio-E	76.4	4.9	nd	0.3	6.6
O2	77.3	5.8	nd	0.5	6.6

Nitrogen analysis results for these pulps are located in Table 16. Laccase HBT and NHAA treated pulps had a slight increase in nitrogen content from 0.01 to 0.02 and 0.03%. Laccase-violuric acid delignified pulp had a much larger increase to 0.09% nitrogen. It appears that nitrogen from the enzyme itself or from mediators is retained in the pulp even after alkaline extraction. This suggests that the mediator or enzyme may be chemically bonded to the fibers. The above viscosity and carbohydrate analysis suggests that the laccase-mediator systems are selective with respect to lignin, so it is likely that the nitrogen is retained in the lignin pulp fraction. The presence of residual mediator chemicals in the pulps may alter the nature of the fiber-fiber bond.

Both HBT and VIO each contain 3 atoms of nitrogen, while NHAA contains only one atom of nitrogen. Because the mediator chemicals were added in equivalent molar concentrations, the mediator contamination of NHAA can be assumed to be three times that of the other mediators at equivalent nitrogen content, assuming the nitrogen comes from mediator sources. Thus the mediator contamination of NHAA is much higher than that of HBT, despite similar nitrogen content.

Table 16: Nitrogen content of kappa 23 (SKP23) pulp following laccase-mediator delignification and alkaline extraction.

Sample	% Nitrogen
Standard deviation	± <0.01
Untreated	<0.01
Untreated-E	0.01
Lhbt-E	0.03
Lnhaa-E	0.02
Lvio-E	0.09
O2	<0.01

PAPER PHYSICAL PROPERTIES

Physical properties of laccase-mediator delignified kappa 23 (SKP23) pulps and one oxygen delignified kappa 23 (SKP23) pulp are shown in the following 16 figures. They are divided into sections in the following order Lhbt-E, Lnhaa-E, Lvio-E, and oxygen delignification for comparison. In each section the order of test measurement is Scott bond, tensile breaking length, MOE, and density. P-values are given for changes in the slope and intercept for each line. A P-value of 0.05 or less indicates a significant difference with 95% or better confidence. In some cases the lines were not curve fitted, but were drawn to include a scattering coefficient value at zero strength.

Lhbt-E

Figures 13-16 show the changes in physical properties of Lhbt-E delignified pulp. As expected from the pulp data, there was no change in Scott bond strength (Figure 13). There was also no change in tensile breaking length (Figure 14), no change in MOE (Figure 15), and no change in density (Figure 16). Delignification of pulp with Lhbt-E appears to have little, if any, effect on pulp physical properties.

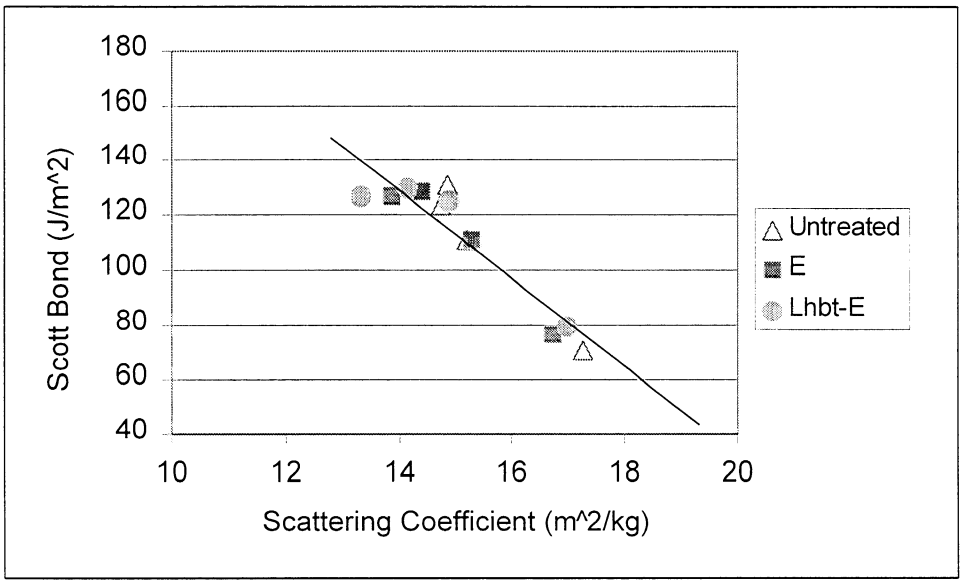

Figure 13: Scott Bond strength versus scattering coefficient for Lhbt-E delignified kappa 23 (SKP23) pulp. There is no statistically significant difference in slope or intercept. P-value for differences in slope = 0.48, P-value for differences in intercept = 0.90.

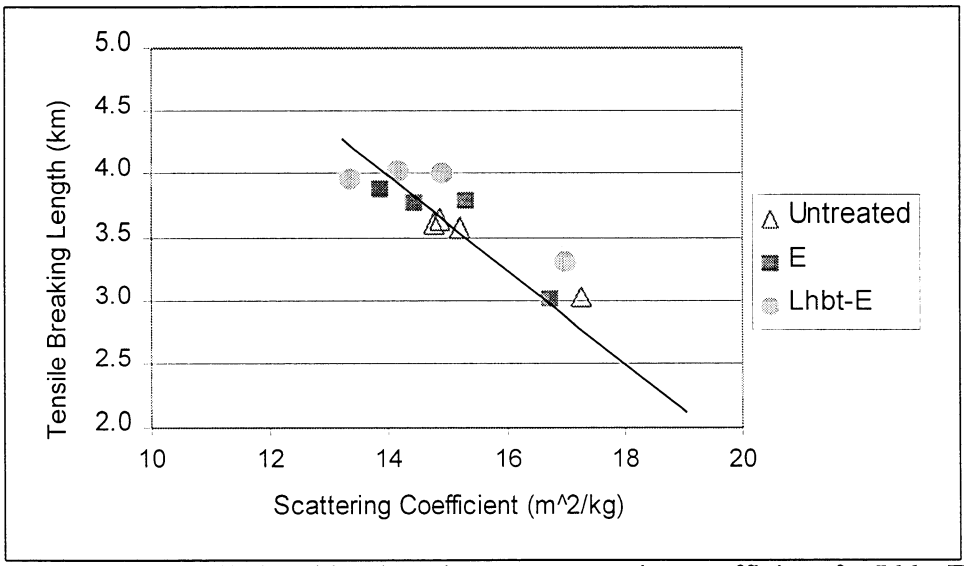

Figure 14: Tensile breaking length versus scattering coefficient for Lhbt-E delignified kappa 23 (SKP23) pulp and untreated pulp. There is no statistically significant difference in slope or intercept. P-value for differences in slope = 0.44, P-value for differences in intercept = 0.30.

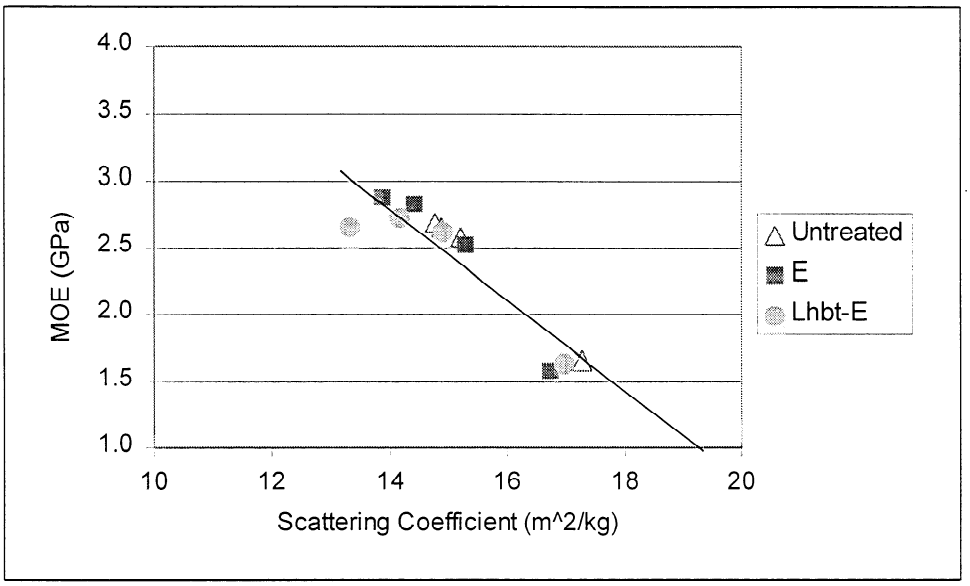

Figure 15: MOE versus scattering coefficient for Lhbt-E delignified kappa 23 (SKP23) pulp. There is no statistically significant difference in slope or intercept. P-value for differences in slope = 0.27, P-value for differences in intercept = 0.46.

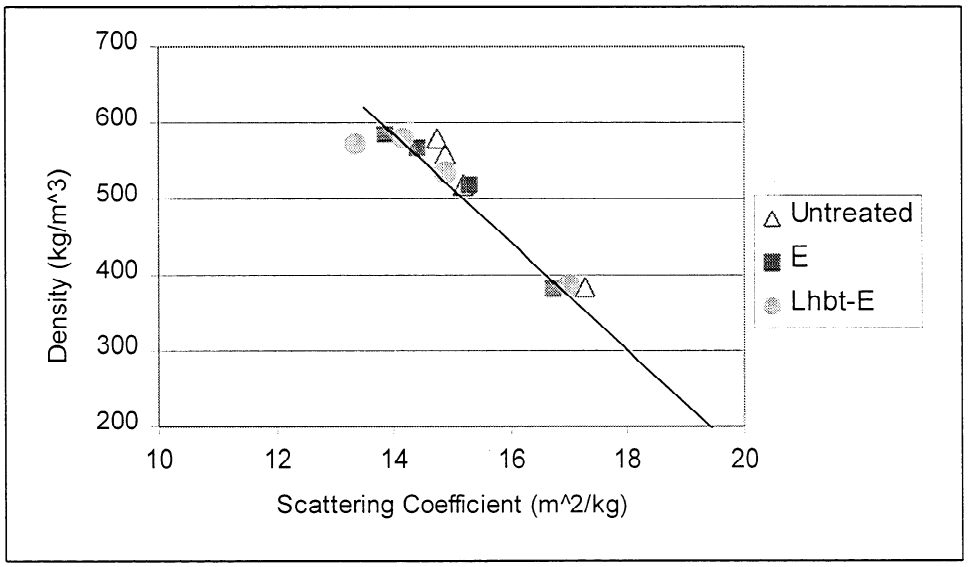

Figure 16: Density versus scattering coefficient for Lhbt-E delignified kappa 23 (SKP23) pulp. There is no statistically significant difference in slope or intercept. P-value for differences in slope = 0.34, P-value for differences in intercept = 0.62.

Lnhaa-E

Figures 17-20 show the physical properties of Lnhaa-E delignified pulps. As expected from the pulp data, there was no change in Scott bond strength (Figure 17) and no change in tensile breaking length (Figure 18). There was a decrease in MOE (Figure 19), and no change in density (Figure 20). Delignification of pulp with Lnhaa-E affects only the MOE of the pulp, and leaves the remaining physical properties unchanged.

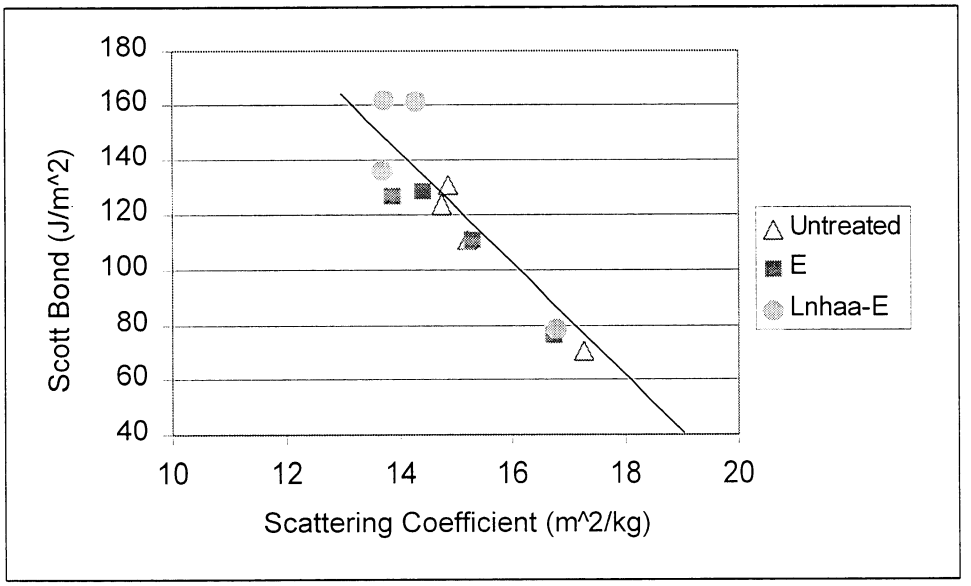

Figure 17: Scott bond versus scattering coefficient for Lnhaa-E delignified kappa 23 (SKP23) pulp. There is no statistically significant difference in slope or intercept. P-value for differences in slope = 0.58, P-value for differences in intercept = 0.22.

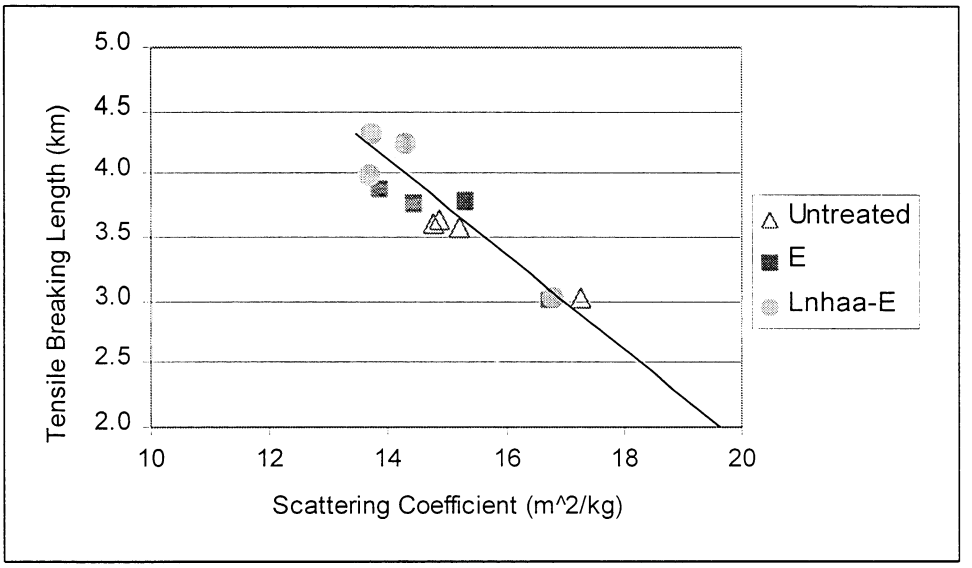

Figure 18: Tensile breaking length versus scattering coefficient for Lnhaa-E delignified kappa 23 (SKP23) pulp. There is no statistically significant difference in slope or intercept. P-value for differences in slope = 0.57, P-value for differences in intercept = 0.43.

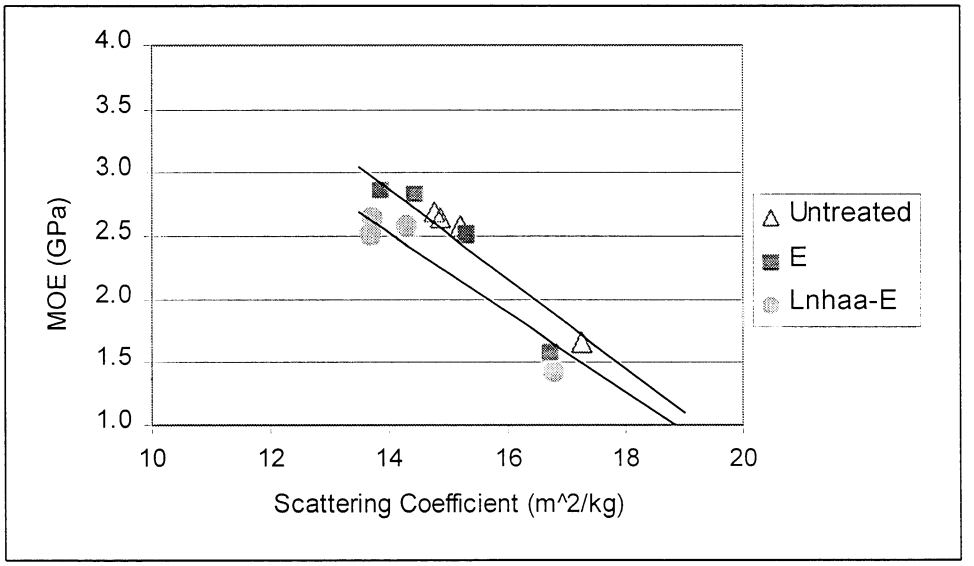

Figure 19: MOE versus scattering coefficient for Lnhaa-E delignified kappa 23 (SKP23) pulp. There is a statistically significant difference (decrease) in intercept. P-value for differences in slope = 0.46, P-value for differences in intercept = 0.029. The upper line connects the untreated alkaline extracted data, and the lower line connects the Lnhaa-E treated data.

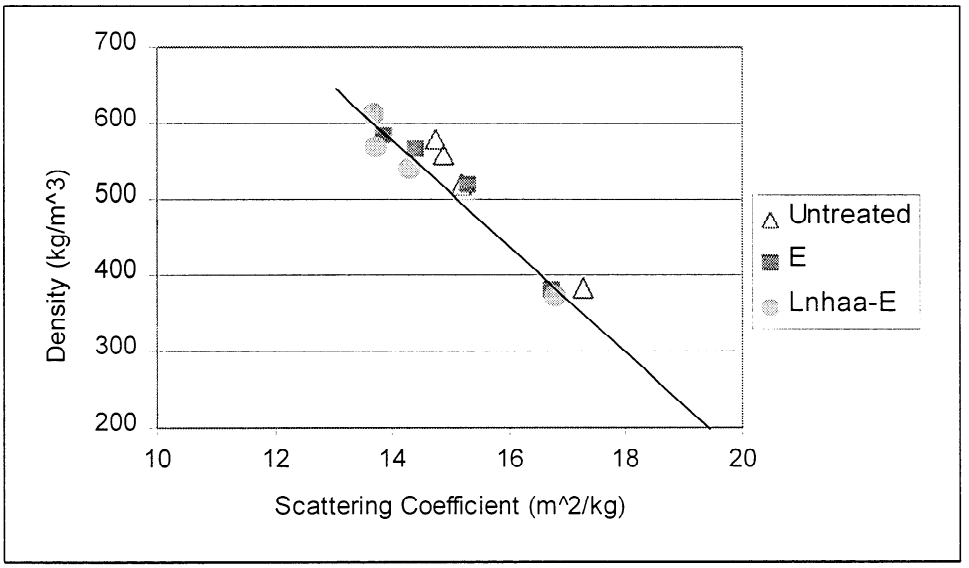

Figure 20: Density versus scattering coefficient for Lnhaa delignified kappa 23 (SKP23) pulp. There is no statistically significant difference in slope or intercept. P-value for differences in slope = 0.93, P-value for differences in intercept = 0.18.

Lvio-E

Figures 21-24 show the physical properties of Lvio-E delignified pulps. Since there was an increase in the surface acid group content as measured by ESCA, an increase in Scott bond strength was expected. There was a significant increase in Scott bond strength for this sample (Figure 21), and an increase in tensile breaking length (Figure 22) associated with the increase in Scott bond strength. There was no change in MOE (Figure 23), and no change in density (Figure 24). Delignification of pulp with Lvio-E appears to increase the bond strength of the resulting paper, and the tensile strength as well.

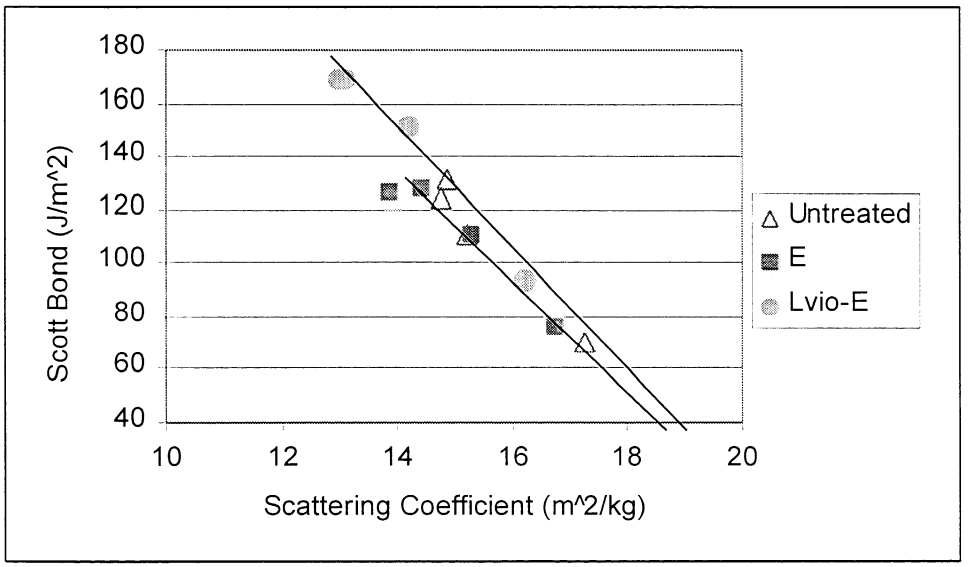

Figure 21: Scott bond strength versus scattering coefficient for Lvio-E delignified kappa 23 (SKP23) pulp. There is a statistically significant difference (increase) in intercept. P-value for differences in slope = 0.26, P-value for differences in intercept = 0.046. The upper line connects the Lvio-E treated data, and the lower line connects the untreated alkaline extracted data.

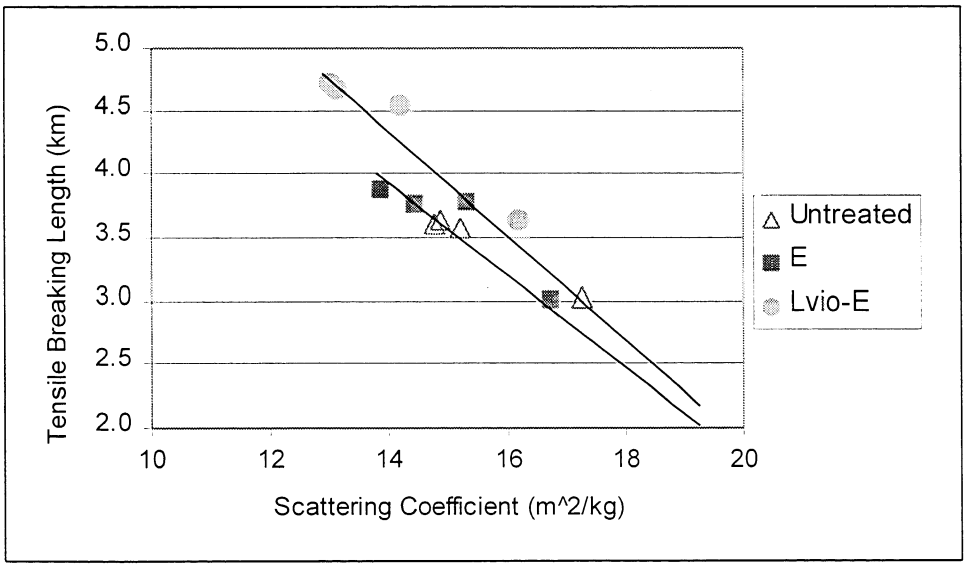

Figure 22: Tensile breaking length for Lvio-E delignified kappa 23 (SKP23) pulp. There is a statistically significant difference (increase) in intercept. P-value for differences in slope = 0.76, P-value for differences in intercept = 0.010. The upper line connects the Lvio-E treated data, and the lower line connects the untreated alkaline extracted data.

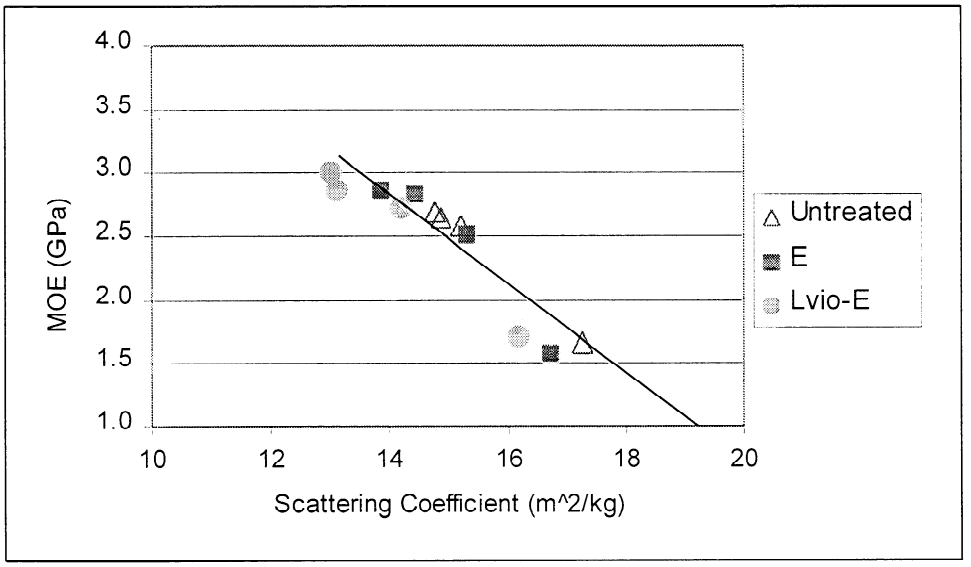

Figure 23: MOE versus light scattering coefficient for Lvio-E delignified kappa 23 (SKP23) pulp. There is no statistically significant difference in slope or intercept. P-value for differences in slope = 0.48, P-value for differences in intercept = 0.073.

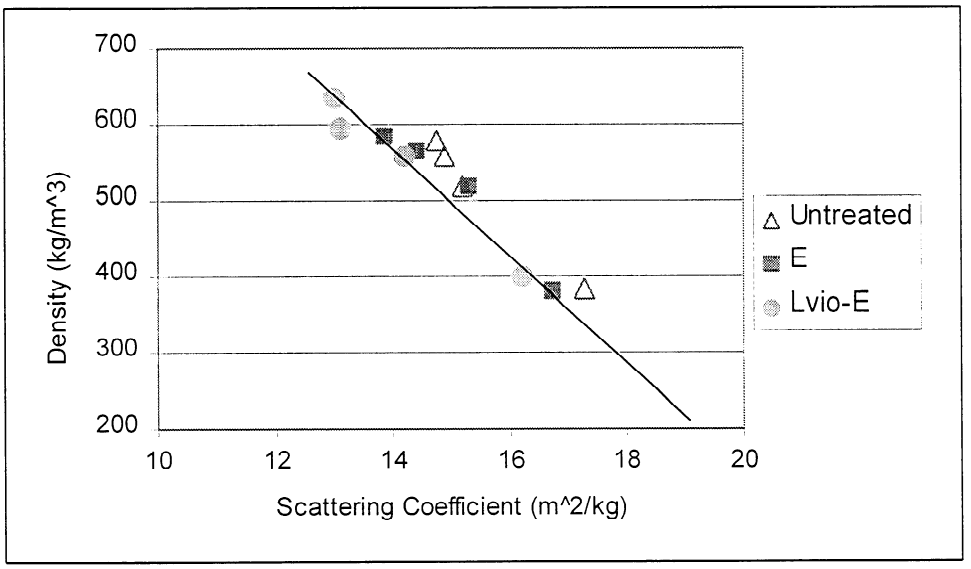

Figure 24: Density versus scattering coefficient for Lvio-E delignified kappa 23 (SKP23) pulp. There is no statistically significant difference in slope or intercept. P-value for differences in slope = 0.80, P-value for differences in intercept = 0.064.

Oxygen

Figures 25-28 show the physical properties of oxygen delignified pulps. Since there was no increase in the surface acid group content as measured by ESCA, no increase in Scott bond strength was expected. However, there was a significant increase in Scott bond strength for this sample (Figure 25) and an increase in tensile breaking length (Figure 26). There was no change in MOE (Figure 27), and no change in density (Figure 28). Delignification of pulp with oxygen delignification appears to increase the bond strength of the resulting paper, and the tensile strength as well. However, the mechanism of this increase does not appear to be due to increased surface acid group content, but rather the removal of lignin.

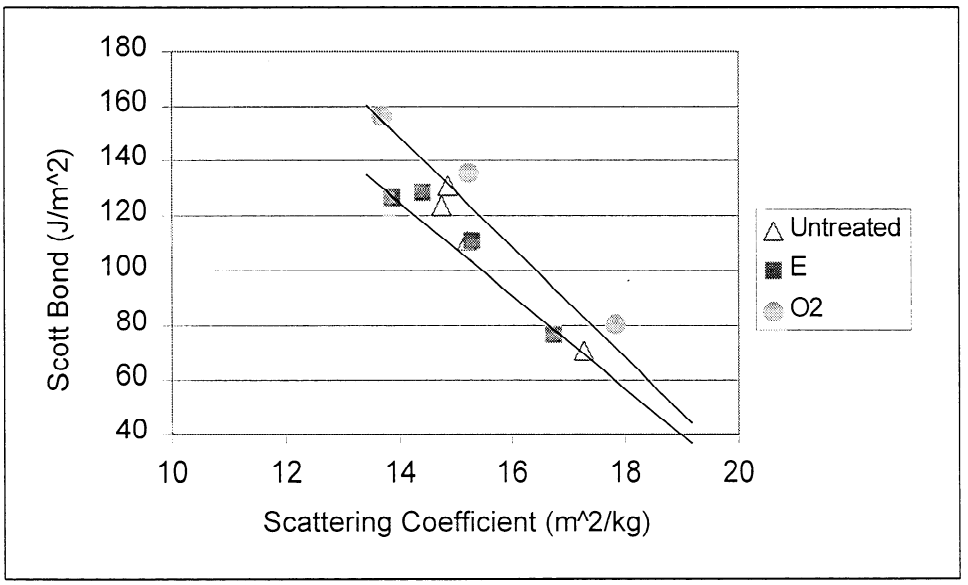

Figure 25: Scott bond versus scattering coefficient for oxygen delignified kappa 23 (SKP23) pulp. There is a statistically significant increase in intercept for the O_2 delignified pulp. P-value for differences in slope = 0.95, P-value for differences in intercept = 0.007. The upper line connects the oxygen treated data, and the lower line connects the untreated alkaline extracted data.

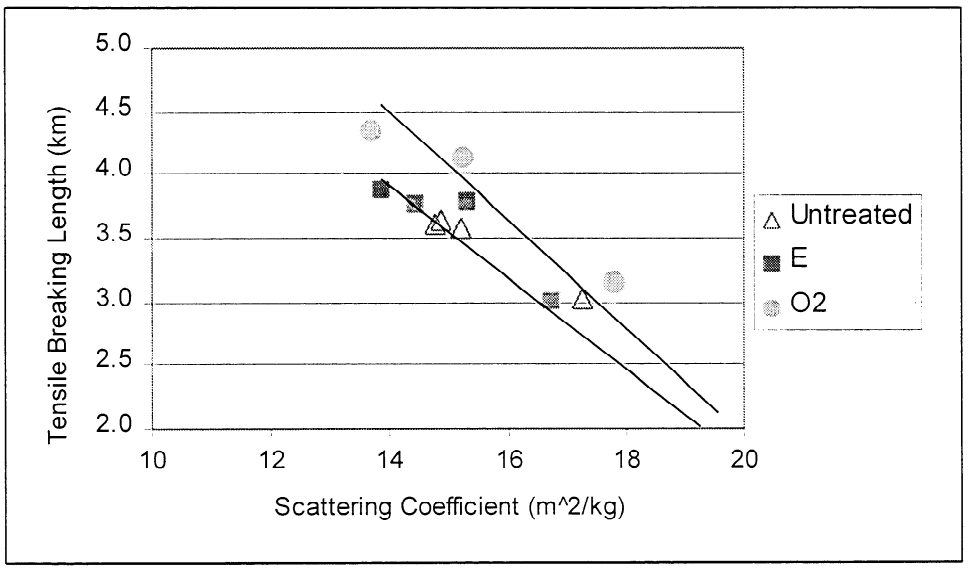

Figure 26: Tensile breaking length versus light scattering coefficient for oxygen delignified kappa 23 (SKP23) pulp. There is a statistically significant difference (increase) in intercept. P-value for differences in slope = 0.98, P-value for differences in intercept = 0.032. The upper line connects the oxygen treated data, and the lower line connects the untreated alkaline extracted data.

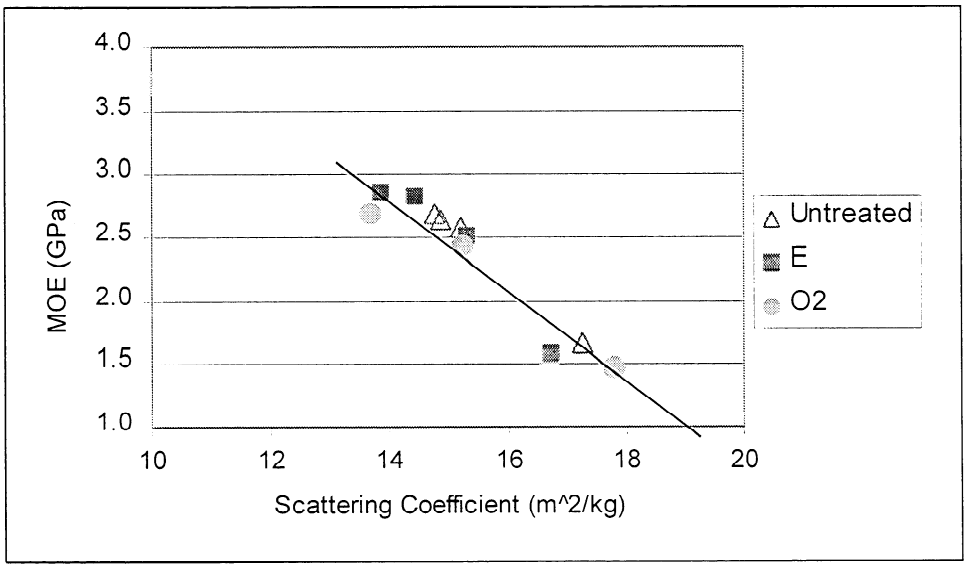

Figure 27: MOE versus scattering coefficient for oxygen delignified kappa 23 (SKP23) pulp. There is no statistically significant difference in slope or intercept. P-value for differences in slope = 0.20, P-value for differences in intercept = 0.68.

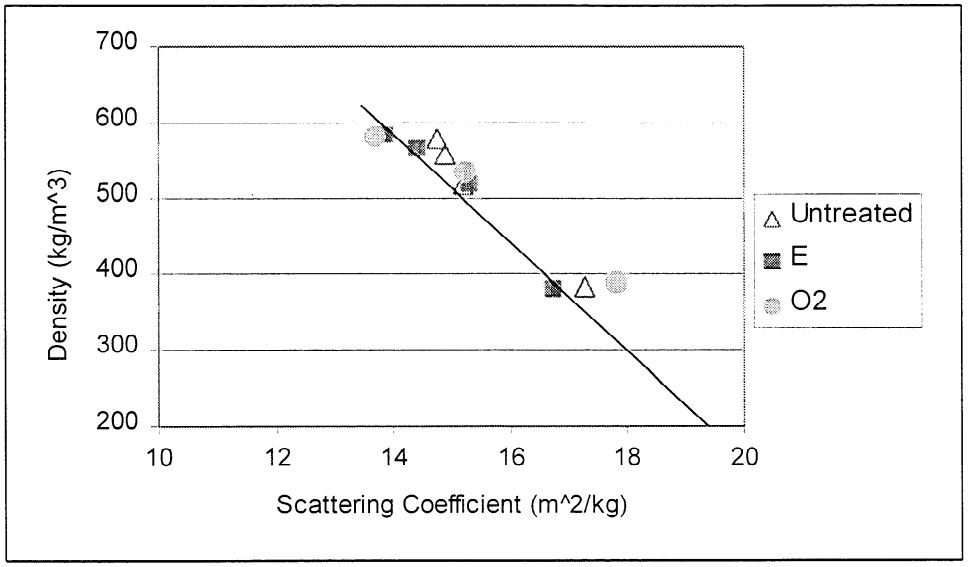

Figure 28: Density versus scattering coefficient for oxygen delignified kappa 23 (SKP23) pulp. There is no statistically significant difference in slope or intercept. P-value for differences in slope = 0.12, P-value for differences in intercept = 0.46.

Comparison between Lvio-E and O_2

A comparison between the Scott bond strength of the Lvio-E and the O_2 pulps was done. The Lvio-E pulp was chosen for this comparison because it was the best system with respect to delignification. The P-values for the comparison between these pulps were 0.22 for the slope and 0.22 for the intercept. There is no difference between the Scott bond strength of the Lvio-E or the O_2 treated pulps.

A similar comparison was run between the tensile breaking lengths for the Lvio-E and the O_2 treated pulps. The P-values for these pulps were 0.73 for the slope and 0.66 for the intercept. There is no statistically significant difference between the tensile breaking lengths of the Lvio-E and O_2 treated pulps.

CONCLUSIONS

Kappa 23 pulp was treated with laccase-mediator systems followed by alkaline extraction, and compared with oxygen extracted pulp. The laccase-violuric acid combination is the most effective of the mediator systems studied at removing lignin from the loblolly pine kraft pulp. The laccase-mediator systems do not decrease viscosity, and are more selective with respect to lignin than oxygen delignification. Oxygen delignification caused a 30% decrease in pulp viscosity. The laccase mediator systems have a negative effect on brightness, and no effect on zero span tensile.

There was a significant increase in surface acid group content (33%) for the Lvio-E delignified pulp, and an increase in Scott bond strength and tensile breaking length. Unexpectedly there was no relationship between surface acid group content and specific bond strength for these lignin containing pulps. There was no increase in surface acid group content for the oxygen treated pulp; however, there was an increase in Scott bond strength and tensile breaking length. The increase in Scott bond strength is probably due to delignification, especially from the fiber surface, rather than acid group content. This result is supported by Laine's ESCA research showing a decrease in lignin content at the fiber surface after oxygen delignification (26,58). The presence of nitrogen contamination indicates enzyme and/or mediator contamination, which may also effect handsheet physical properties. For both Scott bond and tensile breaking length there was no statistically significant difference between the Lvio-E and oxygen delignified pulps.

In this chapter most of this oxidized lignin is removed during the alkaline extraction stage. In order to study the effects of the chemically altered lignin on handsheet physical properties, the alkaline extraction stage must be omitted. The results of laccase-mediator treatment without alkaline extraction are shown in Chapter 2.

CHAPTER 2

PHYSICAL PROPERTIES OF LACCASE-MEDIATOR TREATED KAPPA 23 PULP WITHOUT ALKALINE EXTRACTION

INTRODUCTION

Chapter 1 detailed a thorough investigation into the paper physical properties of laccase mediator delignified kappa 23 pulp. The results from Chapter 1 were compounded by the fact that we removed oxidized lignin during the alkaline extraction stage. In this chapter we would like to study the effects of oxidized lignin on paper properties.

In Chapter 1, there was no observed relationship between the surface carboxylic acid content and the specific bond strength of the paper. Several recent papers support this finding (101,102). Laine (101) measured the surface charge on five softwood and five hardwood pulps using potentiometric titration. He found no relationship between this value and bond strength calculated using the Page equation. However, Laine incorrectly used the Page equation to estimate specific bond strength for pulp where RBA was varied in a beater curve, rather than by wet pressing. Thus, his bond strength results are questionable. In addition his work was done on fully bleached pulp. The acid groups in fully bleached pulp are associated with carbohydrates rather than lignin. The results in Chapters 1 and 2 are for a kappa 23 brownstock kraft starting material. Some of the carboxylic acid groups in this pulp are believed to be present in the lignin fraction. Acid groups in lignin may have a different effect on bonding than acid groups in the carbohydrate fraction.

Robert et al. (102) measured the amount of carboxyl groups in extracted lignin from ozone and chlorine dioxide bleaching stages using ^{13}C NMR. He found no relationship between carboxylic acid groups in lignin and breaking length, although surface acid group concentration was not measured. This work too was not done using wet pressing to vary the RBA.

This chapter is a detailed study of the effects of the presence of laccase-mediator system modified lignin on fiber properties. The surface and bulk acid content of these pulps will help identify any relationship between acid groups in lignin and paper physical properties.

RESULTS AND DISCUSSION

PULP PROPERTIES

Pulp properties of kappa 23 (SKP23) pulps treated with laccase-mediator systems without an alkaline extraction are shown in Table 17. By eliminating the alkaline extraction step, these pulps have retained the reacted lignin. This allows us to study the effects of this reacted lignin material on pulp chemical and physical properties. Table 17 includes measurements of kappa number, viscosity, ESCA analysis of surface carboxylic acids, bulk acid group content, brightness, and zero span tensile. The initial kappa number of the pulp was 23. Treatment with Lhbt caused a slight increase in kappa to 23.9. This may be due to the presence of mediator or enzyme contamination. The mediator HBT may be converted to BT or benzotriazole in delignification reaction. BT has been shown to polymerize and may be deposited on the fibers (3). Lnhaa did not affect the kappa number, while Lvio caused a drop in kappa of 4.6 points, to

18.4. This reduction in kappa number could be caused by the oxidation of lignin during laccase-violuric acid treatment, and may or may not be due to removal of lignin during the laccase-mediator treatment.

There was no significant change in viscosity for any of the treatments. ESCA measurement of surface carboxylic acid groups showed a significant increase for the Lvio treated sample. Bulk acid group content increased for the Lnhaa and Lvio treatments.

Acid insoluble lignin decreased from 4.4 to 3.7 and 3.4%, respectively, for the Lnhaa and Lvio treated samples. The acid insoluble lignin value of 3.4 for Lvio is not less than the acid insoluble lignin value for the alkaline-extracted pulp, despite a lower kappa number. This indicates that the lower kappa number seen for the Lvio treated pulp is probably due to lignin oxidation rather than removal. Acid insoluble lignin did not decrease for the Lhbt sample, and is in good agreement with the kappa results.

Brightness decreased for each of the treated samples, from 21.2 untreated to 17.4 and 17 for Lhbt and Lnhaa and down to 15.6 for Lvio. These results could be due to an increase in darker oxidized lignin material, or to the conversion of mediator chemicals. A decrease in brightness has been reported for both HBT (3) and NHAA (2), and a darkening of the liquor and pulp was visually observed during laccase treatments with all three mediators (3). The zero span tensile results did not change for any of the samples, indicating no change in fiber strength.

Table 17: Pulp properties of kappa 23 (SKP23) pulp treated with laccase-mediator systems, but no alkaline extraction.

Sample	Kappa #	Viscosity (mPa/s)	ESCA COOH (%)	Bulk Acid Content (meq/g)	Acid Insoluble Lignin (%)	Bright-ness (ISO)	Zero Span Tensile (Nm/g)
Standard deviation	±0.3	±0.6	±0.080	±0.006	±0.02	±0.5	±9.5
Untreated	23.0	33.2	0.799	0.063	4.40	21.1	129.7
Untreated-E	21.9	32.8	0.811	0.072	3.40	21.9	126.7
Lhbt	23.9	34.4	0.846	0.071	4.40	17.4	124.3
Lnhaa	23.0	34.0	0.884	0.076	3.70	17.0	124.8
Lvio	18.4	32.1	0.988	0.076	3.40	15.6	128.2

An increase in acid group content was detected for Lvio treated pulp, but not for Lvio-E treated pulp (Chapter 1). This is due to the removal of oxidized lignin material in the alkaline extraction stage. It is clear from this data that carboxylic acid groups are formed in lignin during laccase violuric acid treatment. However the question remains as to whether or not carboxylic acid groups are introduced to the cellulose pulp fraction in the presence of lignin.

Acid chlorite treatments were suggested as a means of answering this question. Both kappa 23 untreated pulp and Lvio-E treated pulp were subjected to acid chlorite liquor. The procedure for this treatment was similar to the acid chlorite treatment done on kappa 50 pulps prior to viscosity measurements, except that the pulps were allowed to sit in the liquor for two days, rather than overnight. Surface and bulk carboxylic acid content were then measured. The results are shown in Table 18. There was no significant increase in either measurement, indicating that carboxylic acid groups are not introduced to the cellulose pulp fraction of the pulp.

Table 18: Surface and bulk acid group content of acid chlorite treated Lvio-E and untreated kappa 23 (SKP23) pulps.

Sample	ESCA COOH (%)	Bulk Acid Content (meq/g)
Untreated	0.682	0.0467
Lvio-E	0.765	0.0406

Carbohydrate analysis of laccase-mediator treated non alkaline-extracted pulps are shown in Table 19. There was no statistically significant change in xylan, galactan, arabinan or mannan content. This suggests that laccase treatment is selective with respect to lignin only.

Table 19: Carbohydrate analysis of kappa 23 (SKP23) non alkaline-extracted pulps. (Standard deviation + 0.5 for xylan and mannan.)

	Glucan %	Xylan %	Galactan %	Arabinan %	Mannan %
Untreated	71.1	5.5	1.0	0.4	6.5
Untreated-E	75.2	5.4	Nd	0.3	6.6
Lhbt	67.1	4.7	Nd	0.5	6.2
Lnhaa	72.6	5.7	Nd	0.6	7.2
Lvio	74.6	4.8	Nd	0.3	6.5

The results of nitrogen analysis of these pulps are shown in Table 20. There was increased nitrogen content for the Lhbt and Lnhaa treated pulps when compared with the untreated pulp. The Lvio treated pulp had the highest increase in nitrogen content from <0.01 to 0.019. The violuric acid mediator is retained in the pulp to a greater degree than the other mediators. In each case, the nitrogen content of these pulps is about twice the nitrogen content of the alkaline extracted pulps in Chapter 1. This indicates that much of the nitrogen is bound to lignin which is removed in the alkaline extraction stage. Again the mediator contamination of

NHAA is three times that of HBT at a given nitrogen content, and in this chapter, is closer to the mediator contamination level of VIO.

Table 20: Nitrogen content of kappa 23 (SKP23) pulp treated with Laccase-mediator systems, but no alkaline extraction.

Sample	% Nitrogen
Standard deviation	± <0.01
Untreated	<0.01
Untreated-E	0.01
Lhbt	0.05
Lnhaa	0.05
Lvio	0.19

PAPER PHYSICAL PROPERTIES

Physical properties of laccase-mediator treated kappa 23 (SKP23) pulps are shown in the following 12 figures. They are divided into sections in the following order Lhbt, Lnhaa, and Lvio. In each section the order of test measurement is Scott Bond, tensile breaking length, MOE, and density. P-values are given for changes in intercept and slope for each line. A P-value of 0.05 or less indicates a significant difference. In some cases the lines were not curve fitted, but were drawn to include a scattering coefficient value at zero strength.

Lhbt

Figures 29-32 show the physical properties for Lhbt treated pulp. There is an increase is Scott bond strength for the Lhbt treated pulp (Figure 29). Since there was no increase in surface acid content, it is clear that a different mechanism is acting to increase the bond strength of this pulp. There was an increase is tensile breaking length (Figure 30) which is the result of the increase in Scott bond strength. There was no change in MOE (Figure 31) or density (Figure 32).

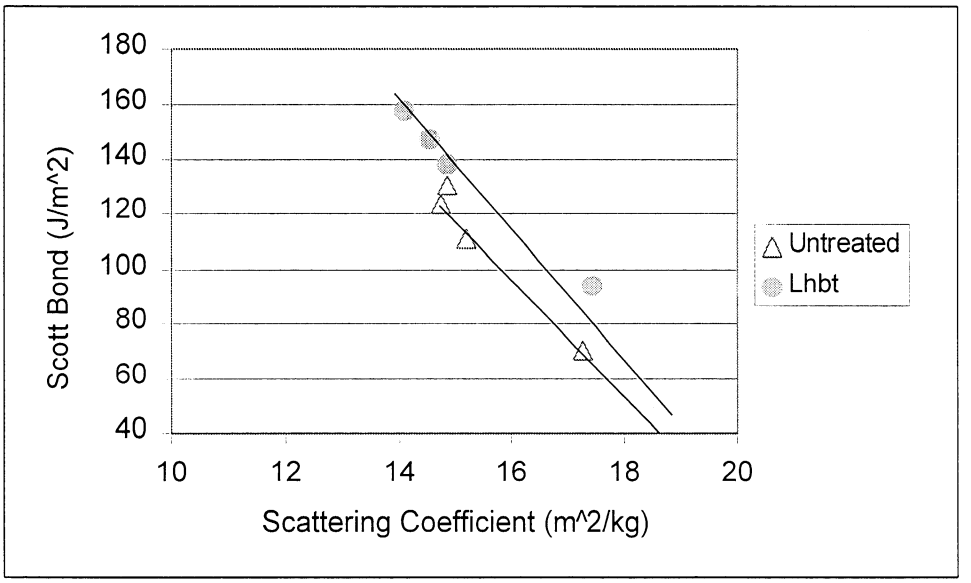

Figure 29: Scott bond strength versus scattering coefficient for Lhbt treated kappa 23 (SKP23) pulp without subsequent alkaline extraction. There is a statistically significant increase in intercept for the Lhbt treated pulp. P-value for differences in slope = 0.26, P-value for differences in intercept = 0.03. The upper line connects the Lhbt treated data, and the lower line connects the untreated data.

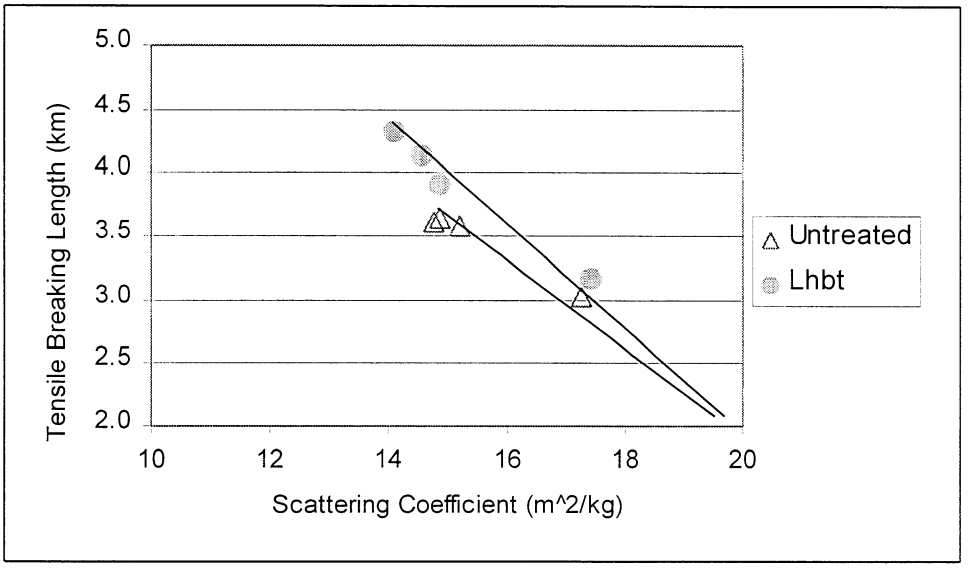

Figure 30: Tensile breaking length versus scattering coefficient for Lhbt treated kappa 23 (SKP23) pulp. There is an increase in intercept for the Lhbt treated pulp. P-value for differences in slope = 0.11, P-value for differences in intercept = 0.02. The upper line connects the Lhbt treated data, and the lower line connects the untreated data.

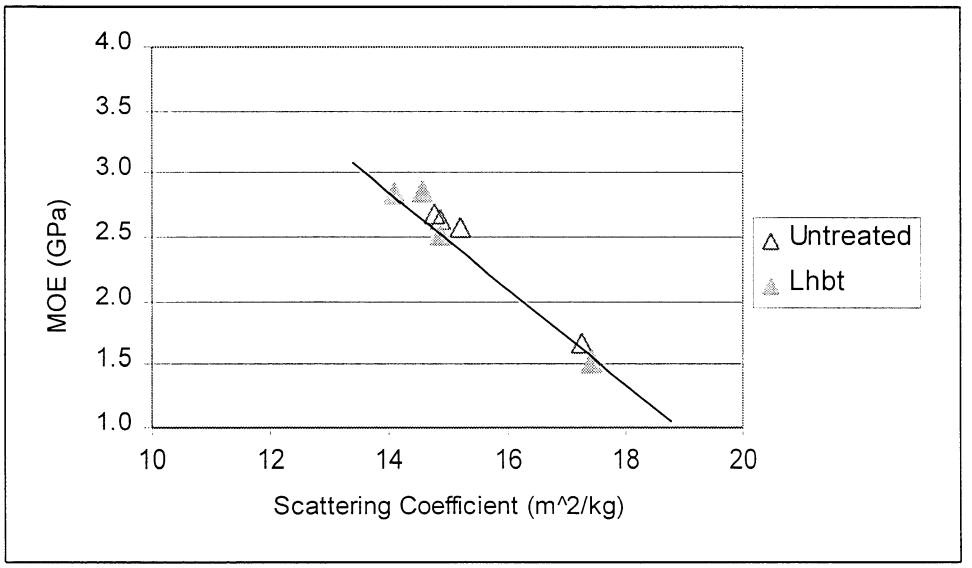

Figure 31: MOE versus scattering coefficient for the Lhbt treated kappa 23 (SKP23) pulp. There is no change in slope or intercept. P-value for differences in slope = 0.96, P-value for differences in intercept = 0.24.

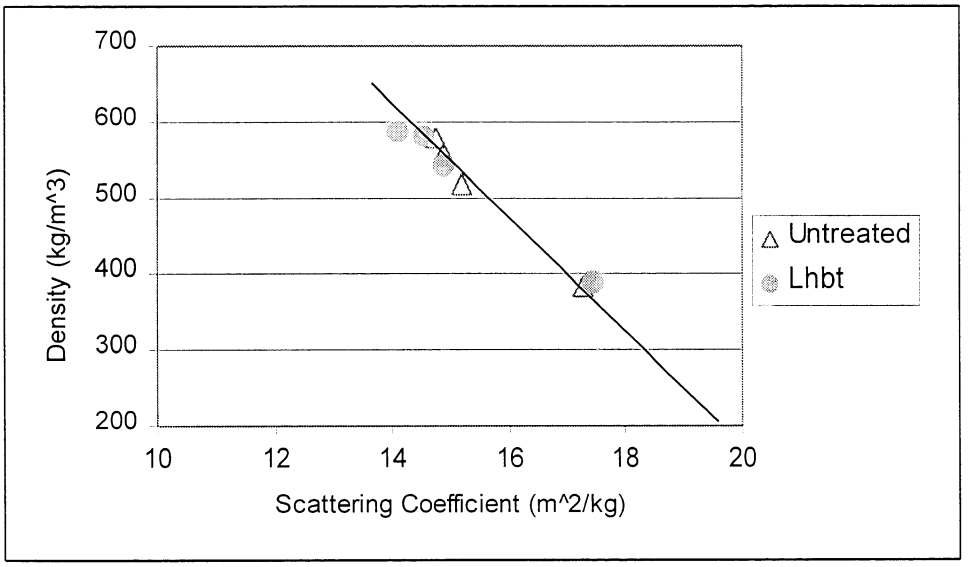

Figure 32: Density versus scattering coefficient for Lhbt treated kappa 23 (SKP23) pulp. There is no change in slope or intercept. P-value for differences in slope = 0.20, P-value for differences in intercept = 0.70.

Lnhaa

Figures 33-36 show the change in pulp physical properties for Lnhaa treated pulp. There was a decrease in Scott bond strength for this pulp (Figure 33), and a decrease in tensile breaking length (Figure 34). There was also a decrease in MOE (Figure 35), and density (Figure 36). Except for a decrease in MOE, the detrimental effect of Lnhaa on pulp physical properties was not seen in Lnhaa-E treated pulps (Chapter 1). The increase in Scott bond strength for the Lhbt treated pulp and the decrease in Scott bond strength for the Lnhaa treated pulp make it unlikely that the same mechanism is at work in both of these samples. This makes enzyme contamination an unlikely source for the changes in paper physical strength properties.

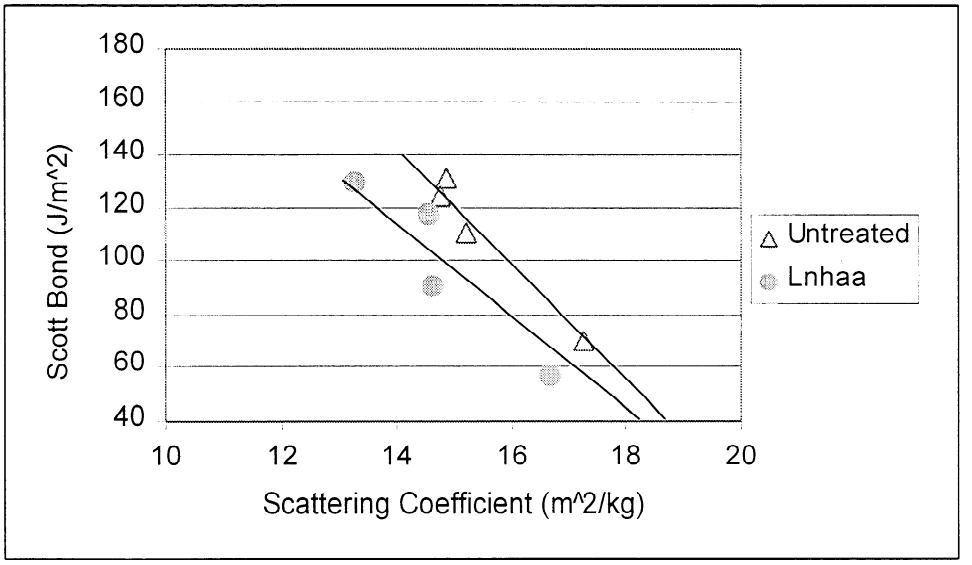

Figure 33: Scott bond strength versus light scattering coefficient for Lnhaa treated kappa 23 (SKP23) pulp. There is an increase in intercept for the Lnhaa treated pulp. P-value for differences in slope = 0.90, P-value for differences in intercept = 0.01. The upper line connects the untreated data, and the lower line connects the Lnhaa treated data.

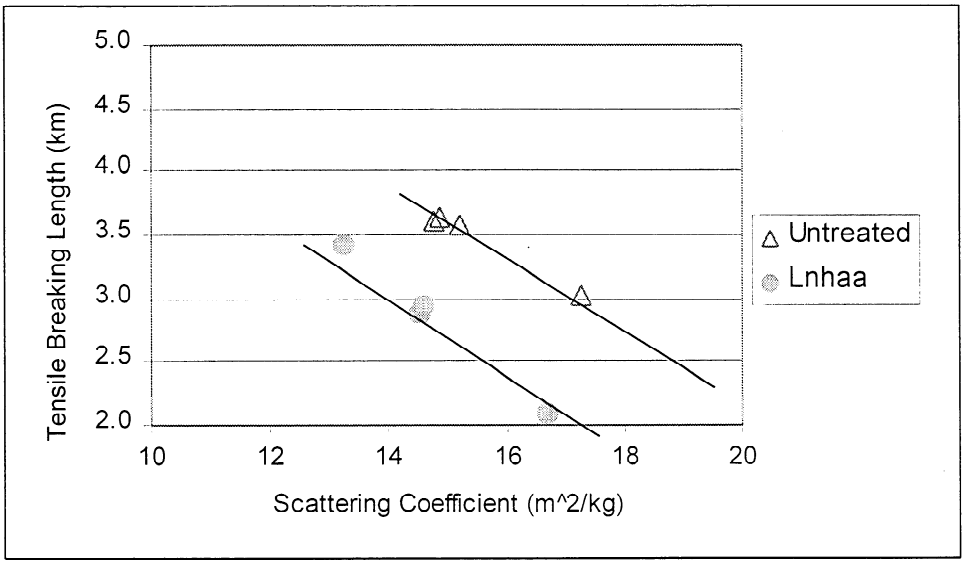

Figure 34: Tensile breaking length versus scattering coefficient for Lnhaa treated kappa 23 (SKP23) pulp. There is a decrease in both intercept and slope for the Lnhaa treated pulp. P-value for differences in slope = 0.007. The upper line connects the untreated data, and the lower line connects the Lnhaa treated data.

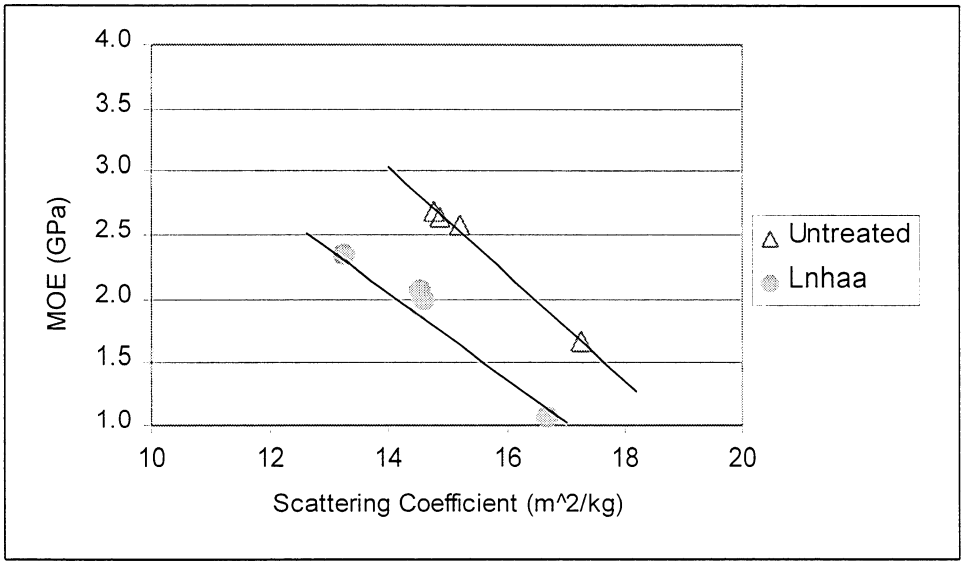

Figure 35: MOE versus scattering coefficient for Lnhaa treated kappa 23 (SKP23) pulp. There is a decrease in slope and intercept for Lnhaa treated pulp. P-value for differences in slope = 0.62, P-value for differences in intercept = 0.00006. The upper line connects the untreated data, and the lower line connects the Lnhaa treated data.

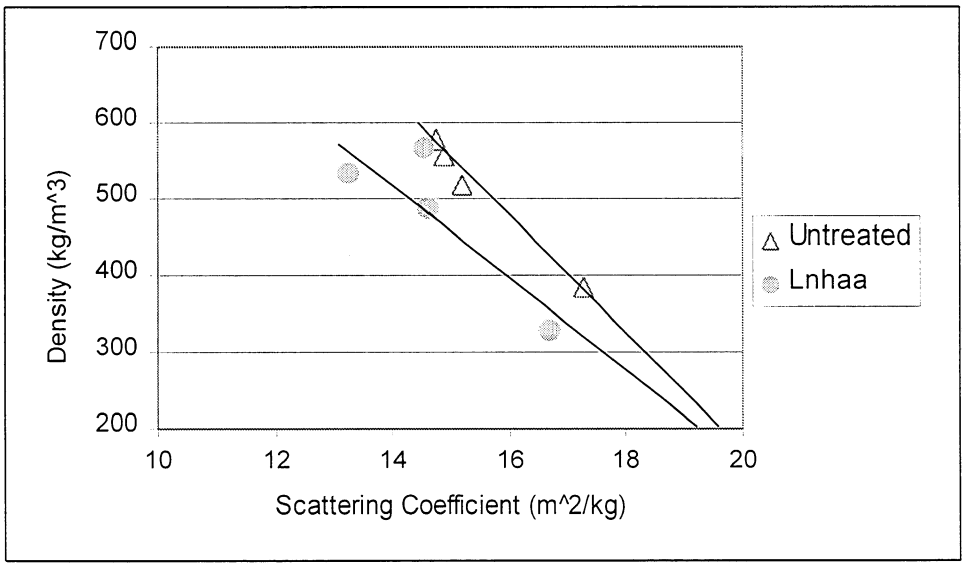

Figure 36: Density versus scattering coefficient for Lnhaa treated kappa 23 (SKP23) pulp. There is a decrease in intercept for the Lnhaa treated pulp. P-value for differences in slope = 0.80, P-value for differences in intercept = 0.045. The upper line connects the untreated data, and the lower line connects the Lnhaa treated data.

Lvio

Figures 37-40 show the change in pulp physical properties for Lvio treated pulp. There was no statistically significant change in Scott bond strength (Figure 37), despite a clear trend toward an increase. An increase in Scott bond strength was anticipated due to the increase in ESCA measurement of surface acid groups. The relationship between acid groups and handsheet properties is complicated for lignin containing pulps, and increases in acid group content do not necessarily cause an increase in Scott bond strength. There was an increase is tensile breaking length (Figure 38), but no change in MOE (Figure 39) or density (Figure 40) for Lvio treated pulp. Mediator contamination may be responsible for the changes in handsheet physical properties for this sample. Again enzyme contamination is an unlikely source for the changes in handsheet physical properties, since the physical properties are different for each mediator tested.

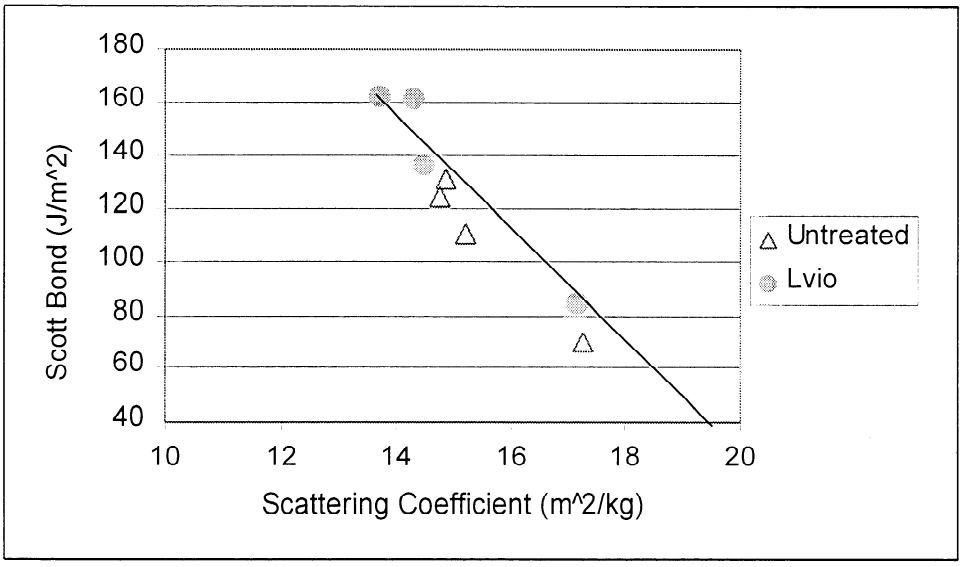

Figure 37: Scott bond strength versus scattering coefficient for the Lvio treated kappa 23 (SKP23) pulp. There is no change in slope or intercept. P-value for differences in slope = 0.93, P-value for differences in intercept = 0.076.

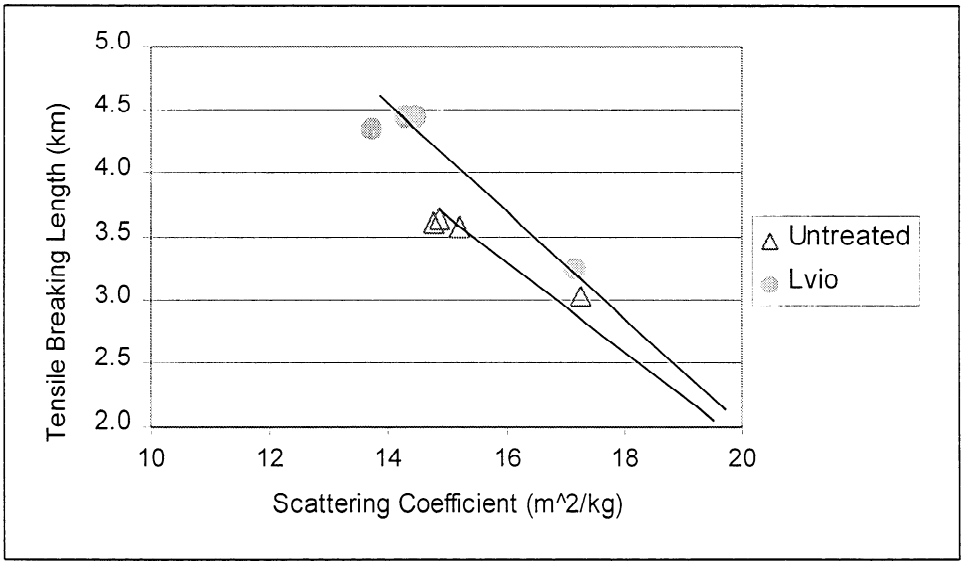

Figure 38: Tensile breaking length versus scattering coefficient for Lvio treated kappa 23 (SKP23) pulp. There is an increase in intercept for the Lvio treated pulp. P-value for differences in slope = 0.28, P-value for differences in intercept = 0.01. The upper line connects the Lvio treated data, and the lower line connects the untreated data.

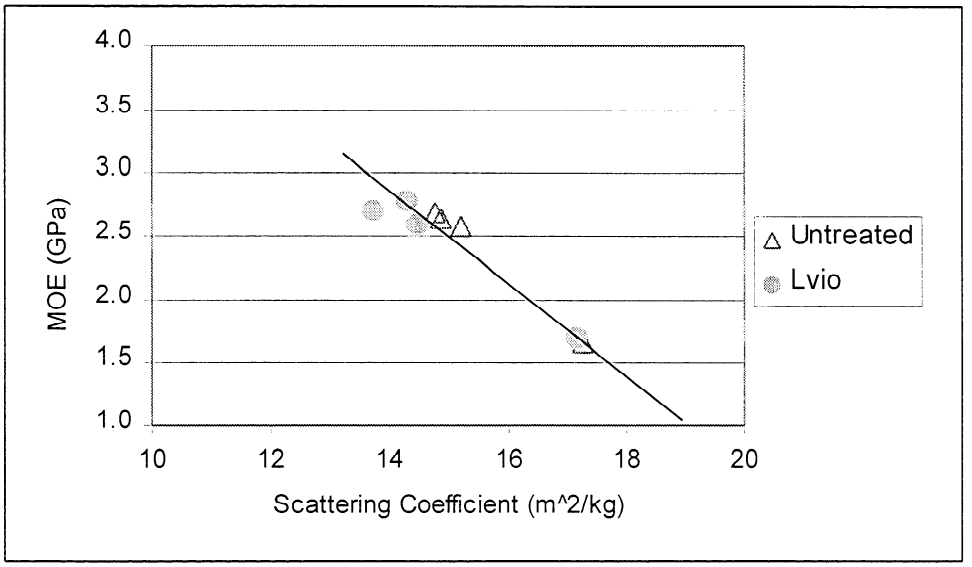

Figure 39: MOE versus scattering coefficient for Lvio treated kappa 23 (SKP23) pulp. There is in change in slope or intercept. P-value for differences in slope = 0.19, P-value for differences in intercept = 0.11.

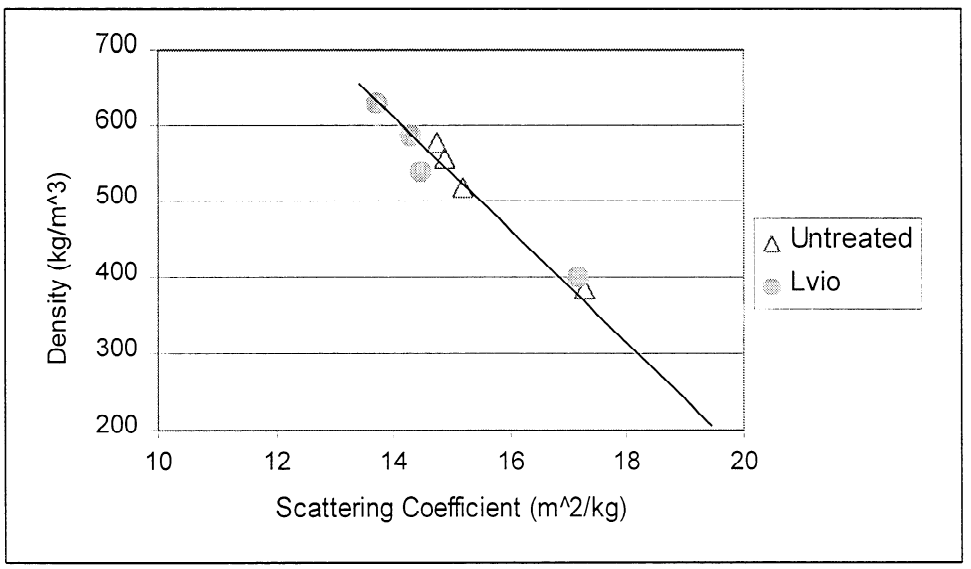

Figure 40: Density versus scattering coefficient for Lvio treated kappa 23 (SKP23) pulp. There is no change in slope or intercept. P-value for differences in slope = 0.44, P-value for differences in intercept = 0.40.

CONCLUSIONS

The pulp properties show that Lvio is effective at oxidizing lignin in kappa 23 loblolly pine pulp, and results in a decrease in kappa number, without a similar decrease in acid insoluble lignin. This indicates there was oxidation of lignin without removal. The laccase-mediator treatments did not affect viscosity or remove hemicellulose, and appears to be selective with respect to lignin. None of the laccase-mediator treatments had any effect on zero span tensile, but all had a negative effect on brightness.

Acid chlorite treatment and subsequent surface and bulk acid group measurement indicated that acid groups were not introduced to the carbohydrate pulp fraction during laccase-violuric acid treatment. In Chapter 6 we will examine the effects of treating fully bleached pulp with laccase-mediator systems. These results differ in that they indicate that acid groups are not introduced to carbohydrates in the presence of lignin.

The results in this chapter allowed us to examine the effects of the oxidized lignin material on handsheet physical properties. Along with the oxidized lignin, there may be some extractive, mediator, reacted mediator species, or enzyme contamination.

There was an increase in Scott bond strength and tensile breaking length for Lhbt, but no increase in surface acid group content. A mechanism of bond strength increase, other than surface acid groups, appears to be acting on this pulp. This increase in specific bond strength could be related to the presence of mediator or reacted mediator species contamination. The presence of the chemically altered lignin or mediator contamination had a beneficial effect on pulp properties for the Lhbt treatment by increasing the hydrophilicity of the lignin, or otherwise improving bonding.

Lnhaa treatment resulted in a decrease in Scott bond strength, tensile breaking length, MOE, and density. There was no decrease in Scott bond strength or tensile breaking length for the kappa 23 Lnhaa-E (alkaline-extracted to remove reacted lignin) pulp in Chapter 1, although there was a decrease in MOE.

There was no statistically significant increase in Scott bond strength for the Lvio treated pulp, despite an increase in ESCA measurement of surface acid groups on lignin. There was a trend toward an increase in Scott bond strength, which is believed to have contributed to the increase in tensile breaking length for this pulp. The increase in handsheet strength properties is due to a mechanism other than the introduction of acid groups.

CHAPTER 3

PHYSICAL PROPERTIES OF LACCASE-MEDIATOR TREATED KAPPA 50 PULP WITHOUT ALKALINE EXTRACTION

INTRODUCTION

In Chapters 1 and 2, the effects of laccase mediator delignification on paper physical properties were examined. The laccase mediator systems appear to introduce acid groups to the lignin fraction of the pulp fibers, and not to the carbohydrate fraction. The influence of acid groups in lignin has been studied by (101,102). They found that acid groups in lignin do not necessarily contribute to bond strength. This finding appears to contradict previous findings (103), where improved paper mechanical properties were attributed to the introduction of carboxyl groups to lignin through ozonation. If acid groups in lignin improve strength properties, then a laccase-mediator treatment might be able to improve the strength properties. This effect should be enhanced by reacting higher kappa pulps with laccase-mediator delignification systems.

Laccase has been investigated as a possible means of modifying high kappa pulp to improve its strength properties (104). One researcher treated a high kappa pulp (kappa 50) with laccase and laccase-HBT (104). Handsheet properties were measured for both non alkaline-extracted and alkaline-peroxide extracted pulps. The results showed that the combination laccase-HBT could increase handsheet density; however, the results in the case of tensile index were mixed. There were several observations of increased tensile strength, and one case where a decrease in the tensile index at a given sheet density was observed (104). Alkaline-peroxide

extraction increased the scattering coefficient, decreased the absorption coefficient, and increased density. However, the alkaline-peroxide extraction did not cause a clear change in handsheet strength properties.

Laccase has been shown to both polymerize and depolymerize lignin (78,105,106). Poppius-Levlin et al. (46) studied the effects of laccase alone and laccase-HBT on lignin isolated from pine kraft pulp using gel permeation chromatography and FTIR. She found that laccase alone easily polymerized small molar mass lignin fragments in solution. Laccase-HBT was effective at delignification, and reduced the molar mass of small molecular weight lignin fragments in solution. She also found an increase in lignin carboxylic acid content after laccase-HBT treatment.

The ability of laccase to introduce carboxylic acid groups to lignin represents an opportunity for the modification of high kappa pulps. It is not clear from the literature whether the introduction of carboxylic acid groups will improve or damage paper physical properties. There is also the possibility for lignin polymerization and deposition on the fiber surface, and for mediator, reacted mediator species, and enzyme contamination of laccase-mediator treated pulps.

This chapter investigates the possibility of modifying high kappa pulps with laccase-mediator treatments. The pulps in this study were not alkaline-extracted, in order to facilitate the study of the effects of the chemically altered lignin and/or mediator structure contamination on paper physical properties.

RESULTS AND DISCUSSION

PULP PROPERTIES

Pulp properties of kappa 50 (SKP50-1) pulps treated with laccase-mediator systems but not with a subsequent alkaline extraction are shown in Table 21. Much of the chemically altered lignin remains with the pulp. This allowed us to identify the effects of this chemically altered lignin on fiber chemical and physical properties. The starting kappa number was 51.2. There was little drop in kappa number for the L, Lhbt, and Lnhaa treatments. The Lhbt-E treatment, which was done for comparison, decreased the kappa number by 20% to 40.3. The Lvio treatment caused a decrease in kappa number of 7 points, to 44.3, without the benefit of alkaline extraction.

Starting viscosity of the brownstock was 41.2cp. The Lvio treatment caused the largest drop in viscosity of 4.5cp to 36.7cp. The remaining treatments caused only a slight decrease in viscosity. Bulk acid group content increased for the L, Lhbt, Lhbt-E, and Lnhaa treated samples. The acid insoluble lignin of the starting material was 8.2 %. All of the treatments caused a decrease in acid insoluble lignin, with Lhbt-E causing the largest decrease. The acid insoluble lignin numbers are in good agreement with the kappa number measurements. The initial ISO brightness of the brownstock was 15.2. All the treatments except Lhbt-E caused a significant decrease in ISO brightness. The lowest ISO brightness (9.9) was obtained by the Lnhaa sample. The ISO brightness decrease was most likely due to mediator or reacted mediator species contamination. There was no significant change in zero span tensile for any of the samples.

Table 21: Pulp properties of kappa 50 (SKP50-1) pulps treated with laccase-mediator systems. No alkaline extraction was done, with the exception of Lhbt-E.

Sample	Kappa #	Viscosity (mPa/s)	ESCA COOH (%)	Bulk Acid Content (meq/g)	Acid Insoluble Lignin (%)	Bright-ness	Zero Span Tensile (Nm/g)
Standard deviation	±0.3	±0.6	±0.08	±0.006	±0.02	±0.5	±9.5
Untreated	51.2	41.2	0.71	0.080	8.20	15.2	123.7
L	50.8	40.3	1.34	0.095	7.30	13.3	134.1
Lhbt	51.0	39.8	1.25	0.101	7.60	13.6	132.5
Lhbt-E	40.3	40.1	1.09	0.098	6.90	15.1	126.7
Lnhaa	48.2	38.7	1.11	0.096	7.90	9.9	124.8
Lvio	44.3	36.7	1.80	0.075	7.20	11.0	133.9

Table 22 contains the carbohydrate analysis data for the kappa 50 (SKP50-1) treated pulps. There were no significant increase or decrease in the carbohydrate analysis, indicating that the laccase is selective with respect to lignin, and does not remove hemicellulose.

Table 22: Carbohydrate analysis of kappa 50 (SKP50-1) treated with laccase-mediator systems. (Standard deviation ± 0.5 for xylan and mannan)

	Glucan %	Xylan %	Galactan %	Arabinan %	Mannan %
Untreated	70.0	4.6	nd	0.6	6.7
L	71.6	4.7	nd	0.2	6.1
Lhbt	71.4	4.8	0.6	0.5	6.9
Lhbt-E	70.1	4.4	nd	0.3	6.5
Lnhaa	72.5	4.6	nd	0.5	6.2
Lvio	71.1	5.1	nd	0.7	7.4

The nitrogen content of these pulps is shown in Table 23. There was an increase in nitrogen content from <0.01 to 0.03% for the laccase only treatment, indicating some contamination from

enzyme sources. The Lhbt and Lnhaa treatments increased the nitrogen content to 0.08. The Lvio treated pulp had 0.23% nitrogen content. The larger increase in nitrogen content of these pulps is believed to be due to the increased lignin content of the kappa 50 pulp. Again the nitrogen content for NHAA and HBT are similar, but the mediator contamination for NHAA is three times that of HBT due to the number of nitrogen atoms present in each molecule.

Table 23: Nitrogen content of kappa 50 (SKP50-1) pulps treated with Laccase-mediator systems. No alkaline extraction was done, with the exception of Lhbt-E.

Sample	% Nitrogen
Standard deviation	± <0.01
Untreated	<0.01
L	0.03
Lhbt	0.08
Lhbt-E	0.06
Lnhaa	0.08
Lvio	0.23

PAPER PHYSICAL PROPERTIES

Physical properties of kappa 50 (SKP50-1) laccase-mediator treated pulps are shown in the following 20 figures. They are divided into sections in the following order: L, Lhbt and Lhbt-E, Lnhaa, and Lvio. In each section, the order of test measurement is Scott bond, tensile breaking length, MOE, and density. P-values are given for changes in slope and intercept for each line. A P-value of 0.05 or less indicates a significant difference. In some cases the lines were not curve fitted, but were drawn to include a scattering coefficient value at zero strength.

Laccase

Figures 41-44 show the physical properties of Laccase (L) treated pulp. There is a decrease in Scott bond strength (Figure 41) and a decrease in tensile breaking length (Figure 42). There is also a decrease in MOE (Figure 43), and density (Figure 44). Both the decrease in Scott bond strength and the decrease in MOE may have contributed to the decrease in tensile breaking length. This indicates that there is sufficient reaction between the laccase and the lignin on the surface of the pulp fiber, without the addition of a mediator, to cause a worsening of paper physical properties. This decrease in handsheet strength properties may be due to precipitated lignin on the surface of the pulp fibers, or to enzyme contamination. Another possibility is that wood extractives may be involved in the laccase reaction with pulp. The possibility of wood extractives playing a role is investigated in Chapter 5.

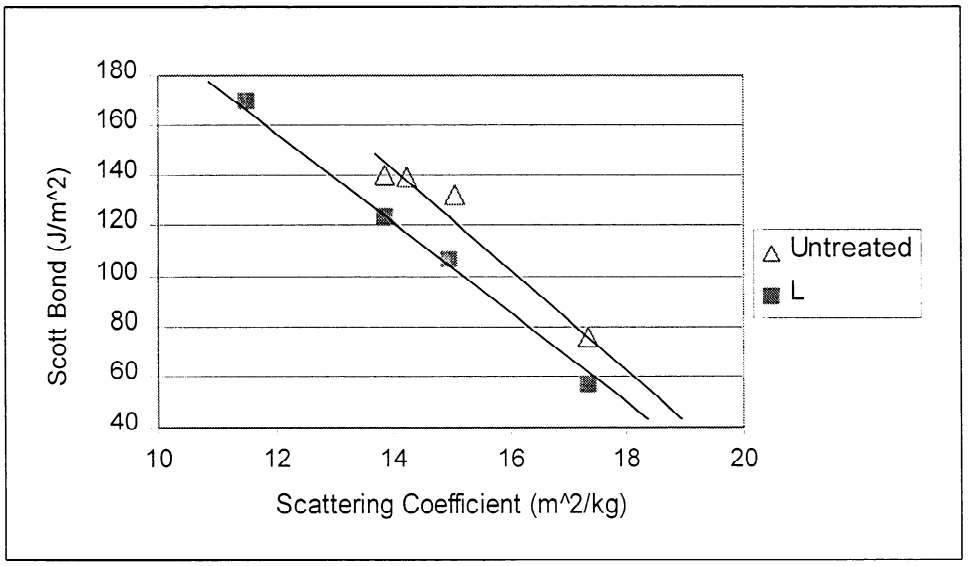

Figure 41: Scott bond strength versus scattering coefficient for L treated kappa 50 (SKP50-1) pulp. There is a decrease in intercept for the L treated pulp. P-value for differences in slope = 0.98, P-value for differences in intercept = 0.0029. The upper line connects the untreated data, and the lower line connects the L treated data.

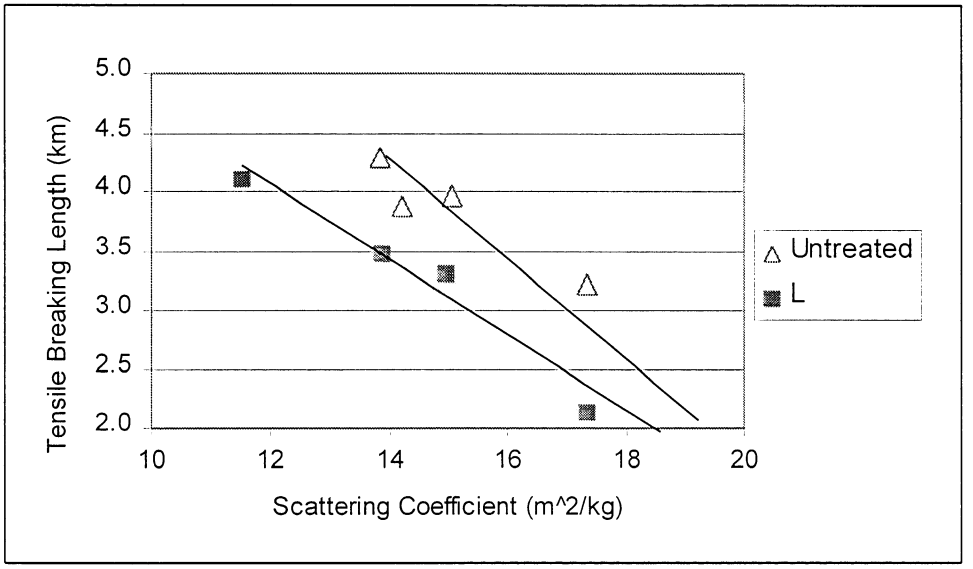

Figure 42: Tensile breaking length versus scattering coefficient for L treated kappa 50 (SKP50-1) pulp. There is a decrease in intercept for the L treated pulp. P-value for differences in slope = 0.55, P-value for differences in intercept = 0.0018. The upper line connects the untreated data, and the lower line connects the L treated data.

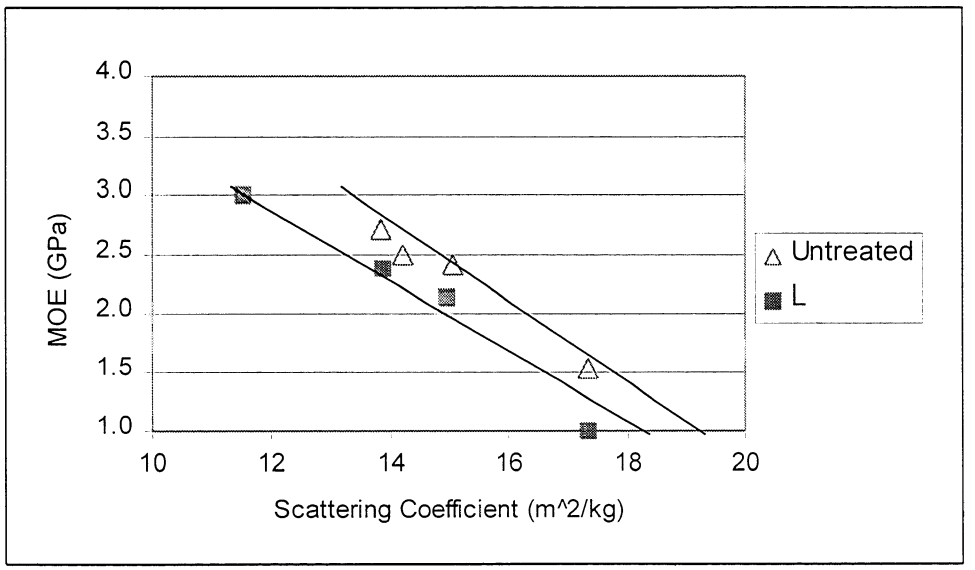

Figure 43: MOE versus scattering coefficient for L treated kappa 50 (SKP50-1) pulp. There is a decrease in intercept for the L treated pulp. P-value for differences in slope = 0.76, P-value for differences in intercept = 0.012. The upper line connects the untreated data, and the lower line connects the L treated data.

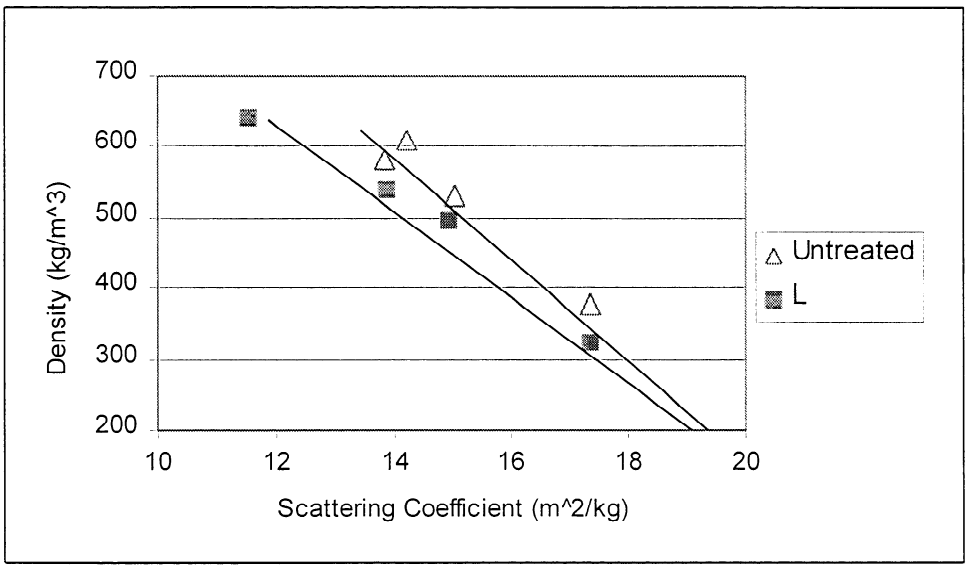

Figure 44: Density versus scattering coefficient for L treated kappa 50 (SKP50-1) pulp. There is a decrease in intercept for the L treated pulp. P-value for differences in slope = 0.41, P-value for differences in intercept = 0.016. The upper line connects the untreated data, and the lower line connects the L treated data.

Lhbt

Figures 45-48 show the physical properties of Lhbt treated pulp. There was no statistically significant difference in the Scott bond strength (Figure 45) despite a clear trend toward a decrease. There was a decrease in tensile breaking length (Figure 46), no change in MOE (Figure 47), and a decrease in density (Figure 48). The trend toward a decrease in Scott bond is believed to be the cause of the decrease in tensile breaking length. Modification of lignin by treating the pulp with Lhbt, and not removing the modified lignin, damages the fiber physical properties. Acid groups in lignin do not increase the specific bond strength for this pulp. There was no decrease in handsheet strength properties for the kappa 23 Lhbt treated pulp in Chapter 2.

Contamination with mediator, reacted mediator species, or enzyme may contribute to the decrease in bonding. The presence of additional lignin in the pulp may affect the reaction between laccase-HBT and pulp, and may affect the polymerization to depolymerization ratios well as the amount of mediator and reacted mediator species present.

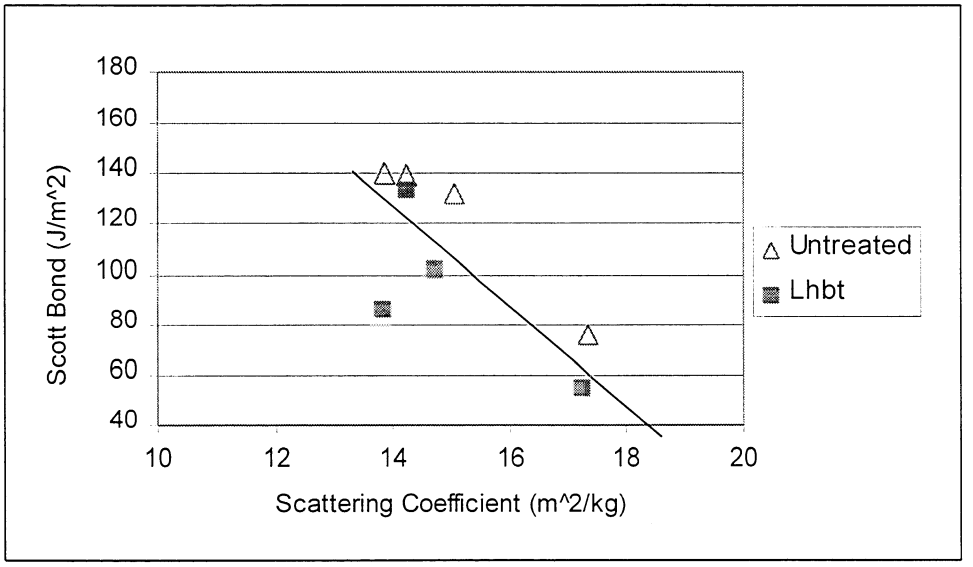

Figure 45: Scott bond strength versus scattering coefficient for Lhbt treated kappa 50 (SKP50-1) pulp. There is no statistically significant change in slope or intercept. P-value for differences in slope = 0.74, P-value for differences in intercept = 0.071. The upper line connects the untreated data, and the lower line connects the Lhbt treated data.

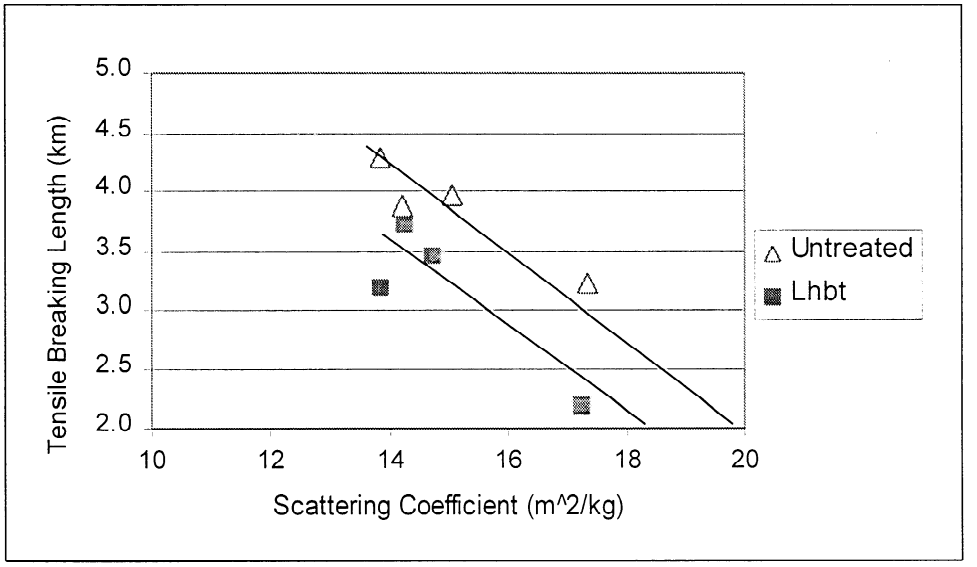

Figure 46: Tensile breaking length versus scattering coefficient for Lhbt treated kappa 50 (SKP50-1) pulp. There is a decrease in intercept for the Lhbt treated pulp. P-value for differences in slope = 0.51, P-value for differences in intercept = 0.015. The upper line connects the untreated data, and the lower line connects the Lhbt treated data.

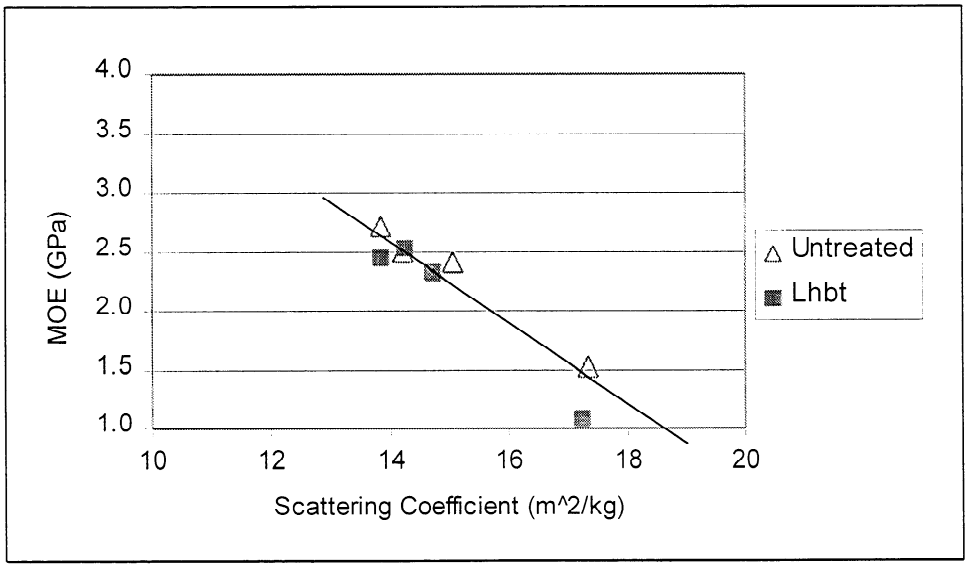

Figure 47: MOE versus scattering coefficient for Lhbt treated kappa 50 (SKP50-1) pulp. There is no change in slope or intercept. P-value for differences in slope = 0.18, P-value for differences in intercept = 0.081. The upper line connects the untreated data, and the lower line connects the Lhbt treated data.

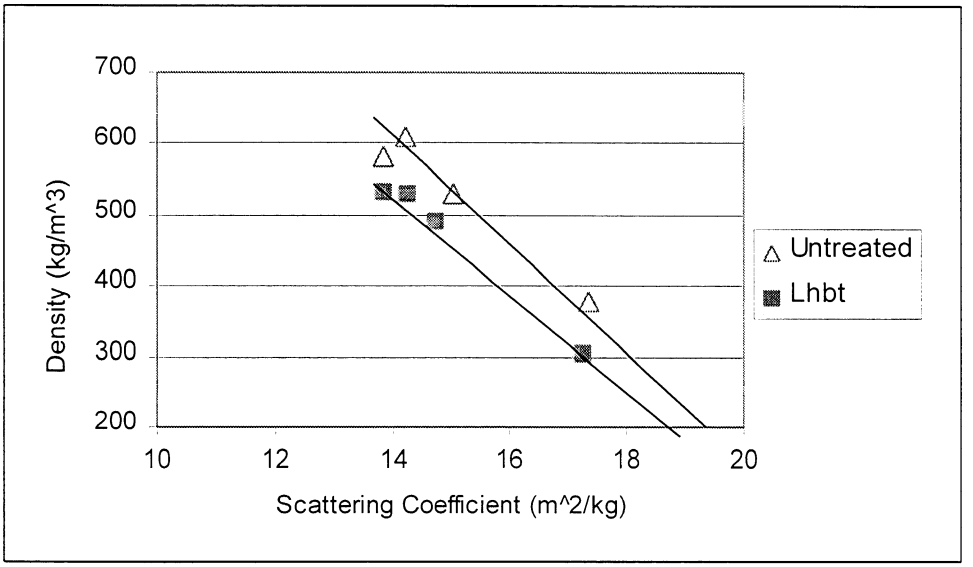

Figure 48: Density versus scattering coefficient for Lhbt treated kappa 50 (SKP50-1) pulp. There is a decrease in intercept for the treated pulp. P-value for differences in slope = 0.60, P-value for differences in intercept = 0.0043. The upper line connects the untreated data, and the lower line connects the Lhbt treated data.

Lhbt-E

Figures 49-52 show the change in paper physical properties for Lhbt-E treated pulp. This pulp sample is the only one in this chapter that was treated using laccase and then subjected to an alkaline extraction stage. This alkaline extraction removed much of the chemically altered lignin. There was a decrease in Scott bond strength (Figure 49), a decrease in tensile breaking length (Figure 50), a decrease in MOE (Figure 51), and a decrease in density (Figure 52). The decrease in Scott bond strength and MOE both contributed to the decrease in tensile breaking length. This is in contrast to the data shown in Chapter 1, where treatment of a kappa 23 (SKP23) pulp had no effect on paper physical properties. The Lhbt-E treatment appears to react differently for the kappa 23 and kappa 50 pulps. This may be due to the increased amount of lignin in the kappa 50 pulp. The additional lignin might affect the polymerization versus depolymerization ratio of lignin.

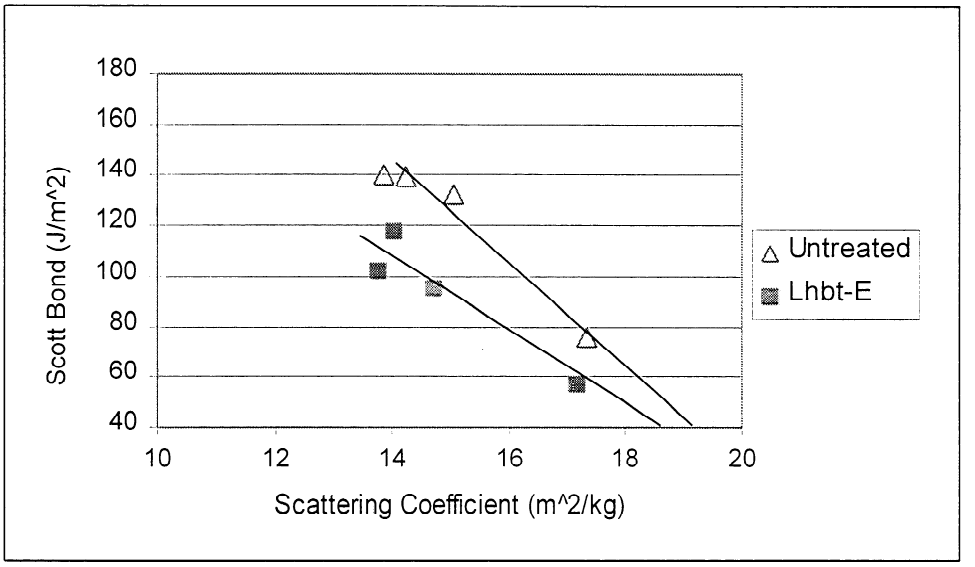

Figure 49: Scott bond strength versus scattering coefficient for Lhbt-E treated kappa 50 (SKP50-1) pulp. There is a decrease in intercept for the Lhbt-E treated pulp. P-value for differences in slope = 0.49, P-value for differences in intercept = 0.0031. The upper line connects the untreated data, and the lower line connects the Lhbt-E treated data.

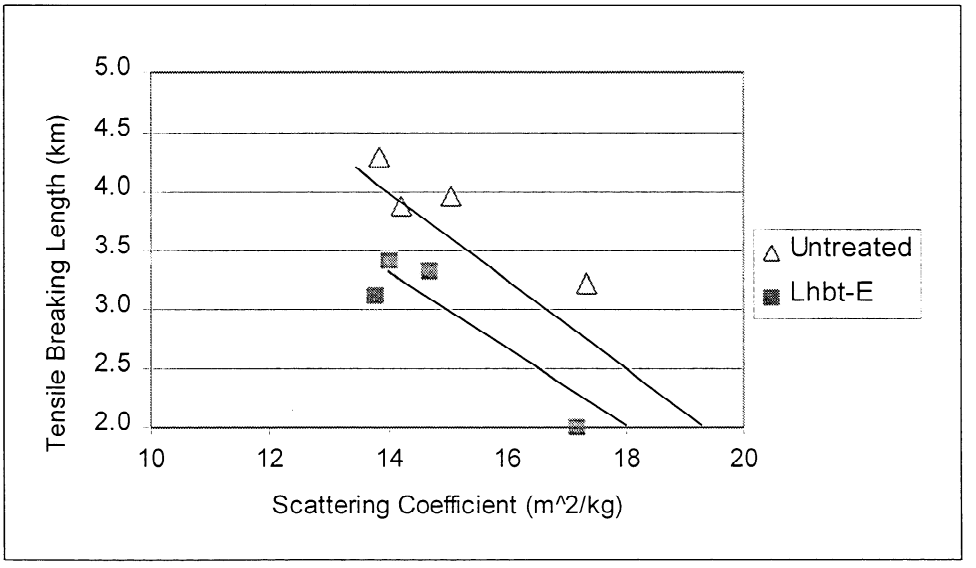

Figure 50: Tensile breaking length versus scattering coefficient for Lhbt-E treated kappa 50 (SKP50-1) pulp. There is a decrease in intercept for the Lhbt-E treated pulp. P-value for differences in slope = 0.40, P-value for differences in intercept = 0.0031. The upper line connects the untreated data, and the lower line connects the Lhbt treated data.

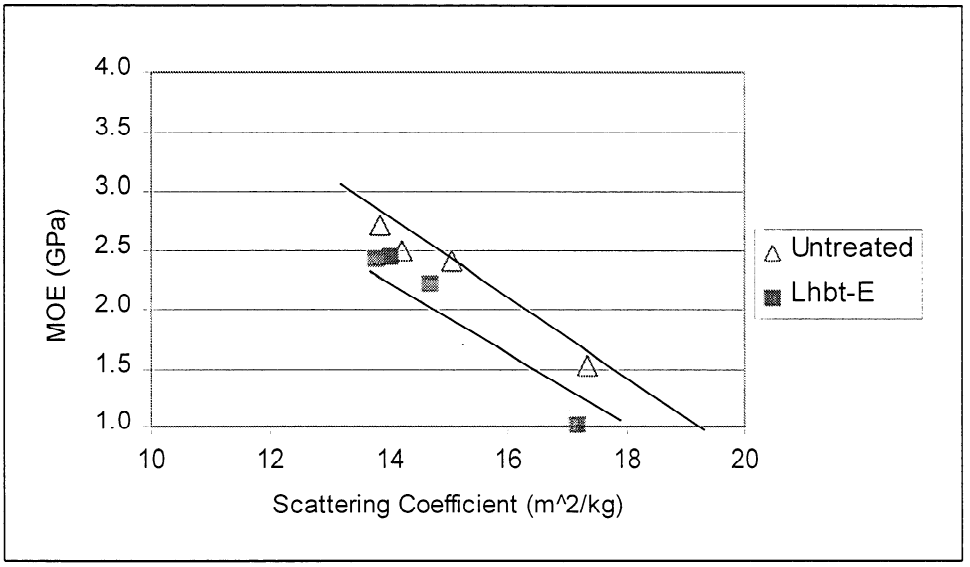

Figure 51: MOE versus scattering coefficient for the Lhbt-E treated kappa 50 (SKP50-1) pulp. There is a decrease in intercept for the Lhbt-E treated pulp. P-value for differences in slope = 0.10, P-value for differences in intercept = 0.013. The upper line connects the untreated data, and the lower line connects the Lhbt treated data.

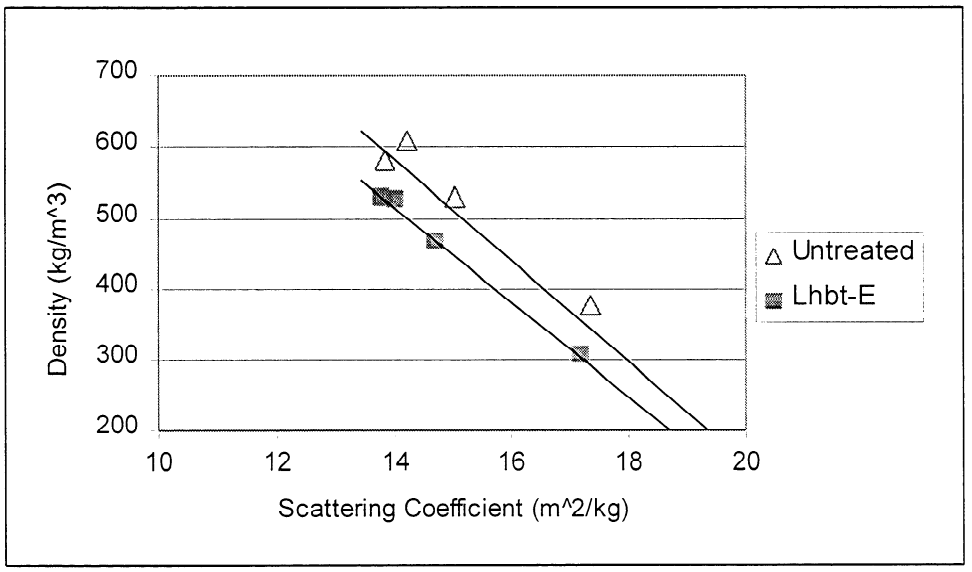

Figure 52: Density versus scattering coefficient for the Lhbt-E treated kappa 50 (SKP50-1) pulp. There is a decrease in intercept for the Lhbt-E treated pulp. P-value for differences in slope = 0.82, P-value for differences in intercept = 0.0012. The upper line connects the untreated data, and the lower line connects the Lhbt treated data.

Lnhaa

Figures 53-56 show the changes in paper physical properties for Lnhaa treated pulp. There was a decrease in Scott bond strength (Figure 53), a decrease in tensile breaking length (Figure 54), a decrease in MOE (Figure 55), and a decrease in density (Figure 56). Lnhaa treatment has a negative affect on paper physical properties. This trend toward a decrease in handsheet strength properties was the same for Lhbt treatment, and may be due to polymerized lignin on the surface of the pulp, or to mediator or enzyme contamination. The mediator NHAA appears to cause a decrease in MOE, both in this chapter and in Chapters 1 and 2. This is probably due to a change in the nature of the oxidized lignin material in the pulp.

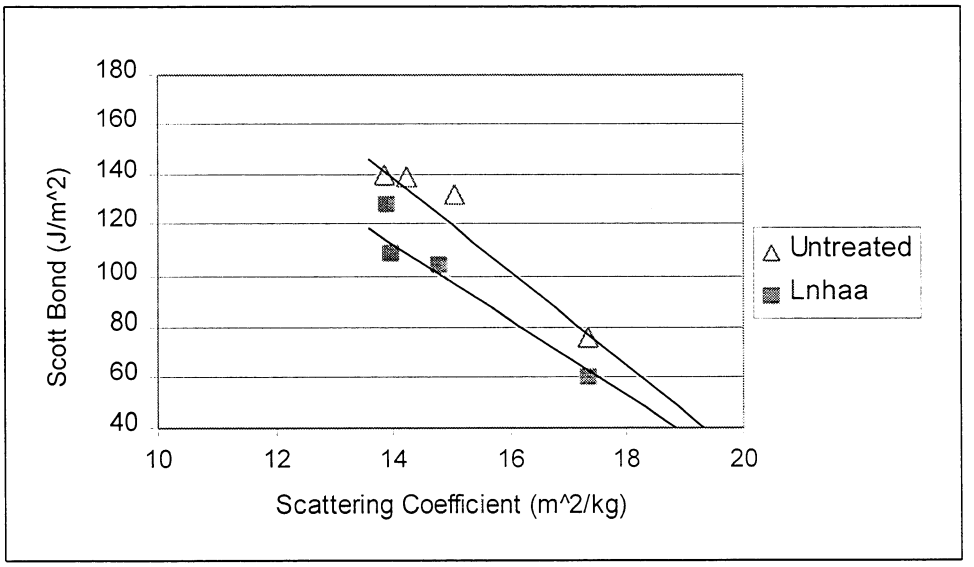

Figure 53: Scott bond strength versus scattering coefficient for the Lnhaa treated kappa 50 (SKP50-1) pulp. There is a decrease in intercept for the Lnhaa treated pulp. P-value for differences in slope = 0.66, P-value for differences in intercept = 0.0086. The upper line connects the untreated data, and the lower line connects the Lnhaa treated data.

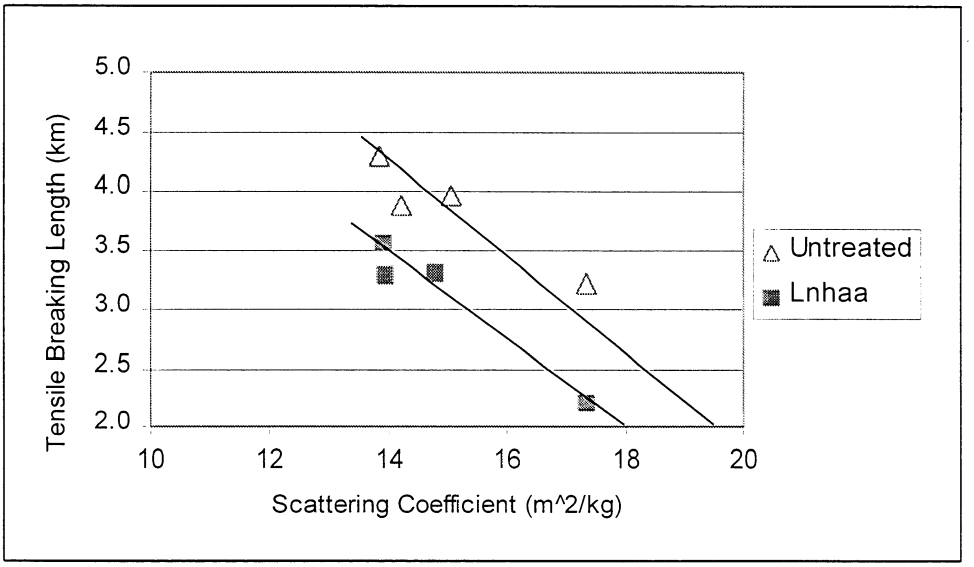

Figure 54: Tensile breaking length versus scattering coefficient for the Lnhaa treated kappa 50 (SKP50-1) pulp. There is a decrease in intercept for the Lnhaa treated pulp. P-value for differences in slope = 0.40, P-value for differences in intercept = 0.0013. The upper line connects the untreated data, and the lower line connects the Lnhaa treated data.

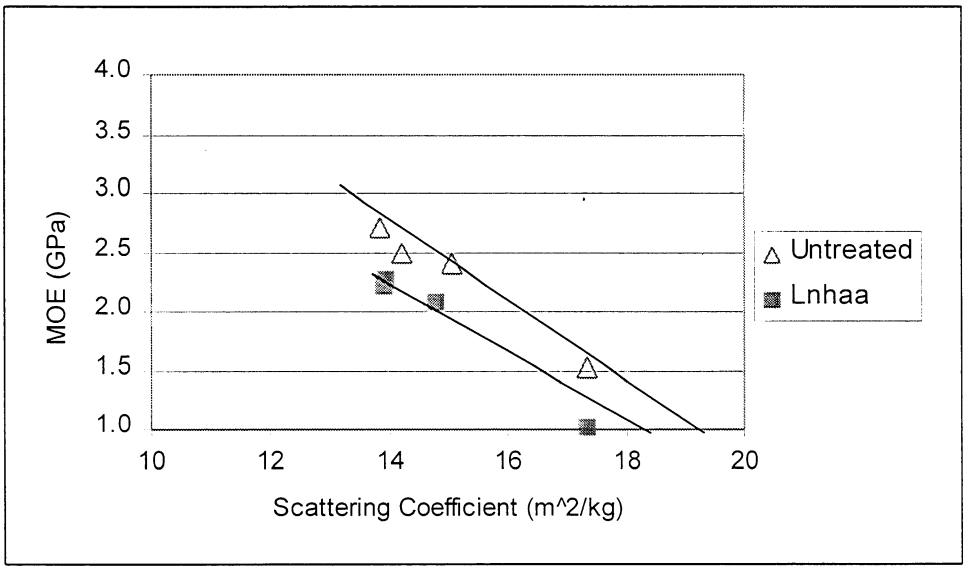

Figure 55: MOE versus scattering coefficient for the Lnhaa treated kappa 50 (SKP50-1) pulp. There is a decrease in intercept for the Lnhaa treated pulp. P-value for differences in slope = 0.54, P-value for differences in intercept = 0.0014. The upper line connects the untreated data, and the lower line connects the Lnhaa treated data.

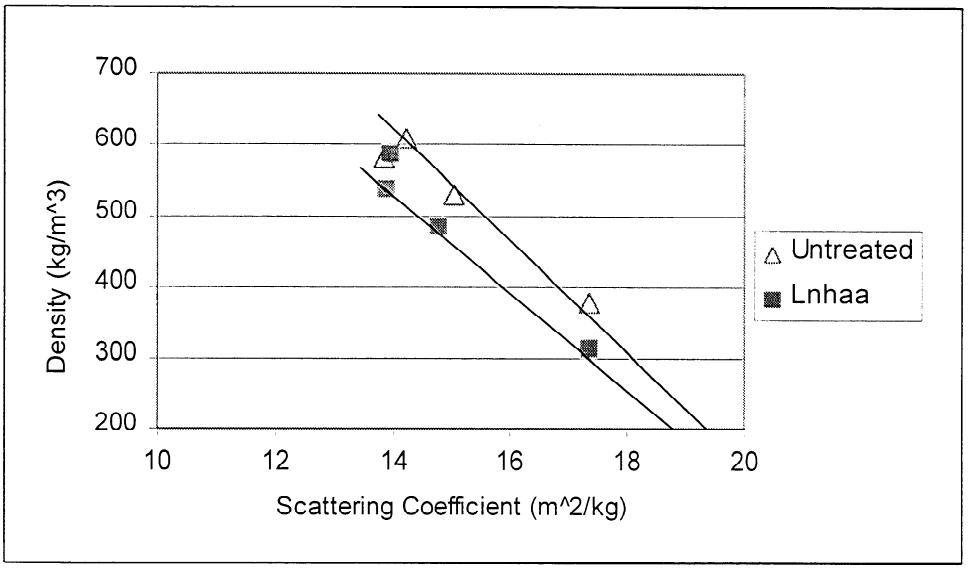

Figure 56: Density versus scattering coefficient for the Lnhaa treated kappa 50 (SKP50-1) pulp. There is a decrease in intercept for the Lnhaa treated pulp. P-value for differences in slope = 0.61, P-value for differences in intercept = 0.032. The upper line connects the untreated data, and the lower line connects the Lnhaa treated data.

Lvio

Figures 57-60 show the paper physical properties for Lvio treated pulp. There was a decrease in Scott bond strength (Figure 57), a decrease in tensile breaking length (Figure 58), and a decrease in MOE (Figure 59). There was no significant difference in density (Figure 60). The decrease in tensile breaking length is due to the decrease in Scott bond strength and the decrease in MOE. The lignin left in the fiber after Lvio treatment has a negative effect on paper physical properties, which again agrees with the trend toward a decrease in handsheet strength properties for Lhbt and Lnhaa treated pulps.

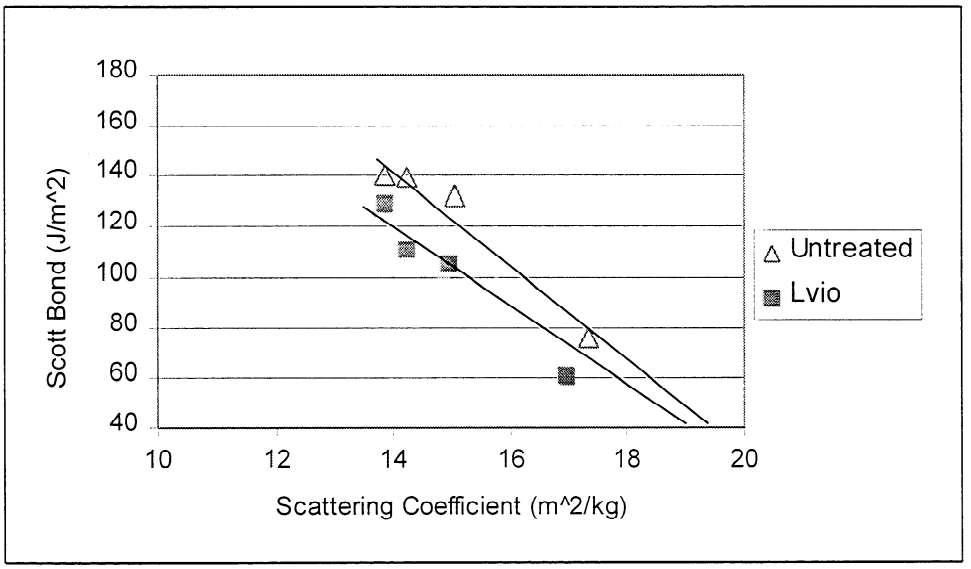

Figure 57: Scott bond strength versus scattering coefficient for the Lvio treated kappa 50 (SKP50-1). There is a decrease in intercept for the Lvio treated pulp. P-value for differences in slope = 0.76, P-value for differences in intercept = 0.0045. The upper line connects the untreated data, and the lower line connects the Lvio treated data.

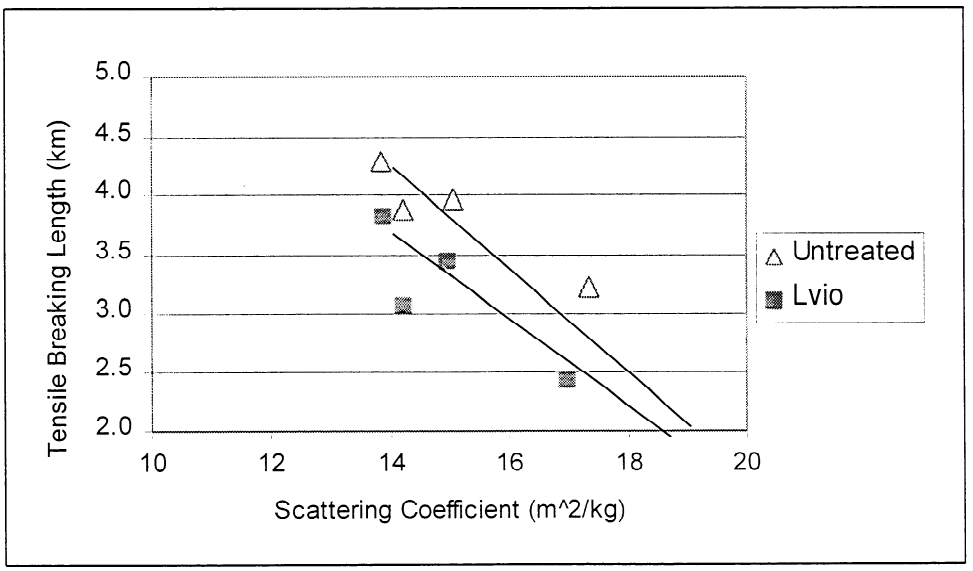

Figure 58: Tensile breaking length versus scattering coefficient for the Lvio treated kappa 50 (SKP50-1) pulp. There is a decrease in intercept for the Lvio treated pulp. P-value for differences in slope = 0.56, P-value for differences in intercept = 0.016. The upper line connects the untreated data, and the lower line connects the Lvio treated data.

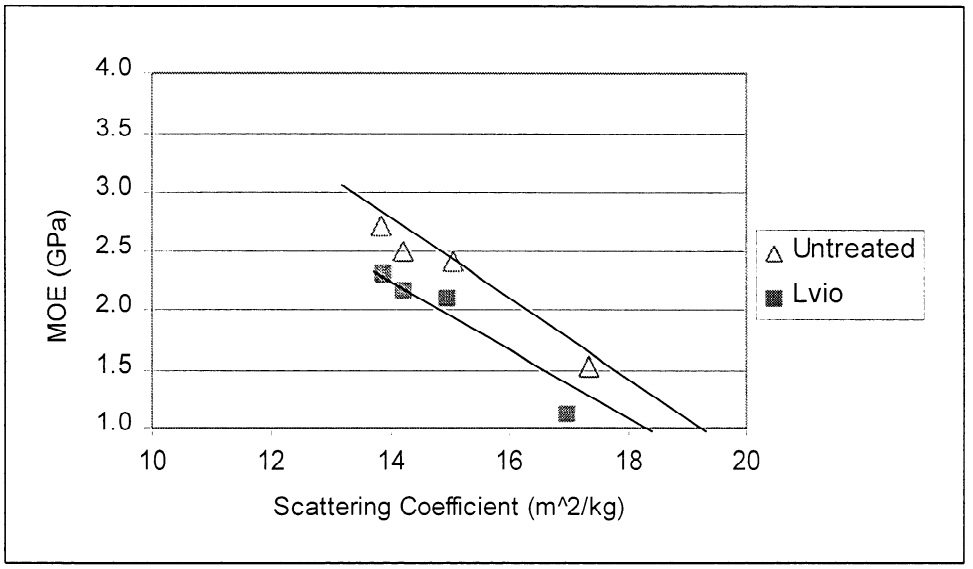

Figure 59: MOE versus scattering coefficient for the Lvio treated kappa 50 (SKP50-1) pulp. There is a decrease in intercept for the Lvio treated pulp. P-value for differences in slope = 0.41, P-value for differences in intercept = 0.0036. The upper line connects the untreated data, and the lower line connects the Lvio treated data.

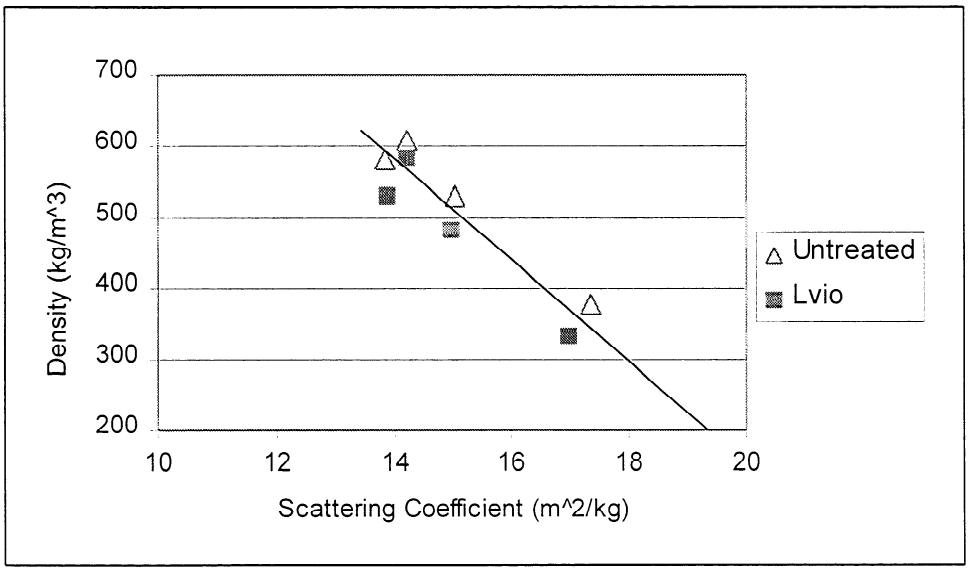

Figure 60: Density versus scattering coefficient for the Lvio treated kappa 50 (SKP50-1) pulp. There is no change in slope or intercept. P-value for differences in slope = 0.64, P-value for differences in intercept = 0.07. The upper line connects the untreated data, and the lower line connects the Lvio treated data.

CONCLUSIONS

It was hoped that modification of kappa 50 pulps with laccase-mediator systems might provide an increase in strength properties through surface carboxylic acid addition. Unfortunately, the treatment of kappa 50 pulp with the laccase-mediator systems has a negative effect on paper physical properties in all cases. Even when the pulp was subjected to an alkaline extraction stage to remove much of the modified lignin (Lhbt-E), there was still a decrease in physical properties associated with the treatment. Apparently oxidation of the carbohydrate pulp fraction is necessary to get an improvement in specific bond strength.

There was a decrease in the density of each of the pulps. The decrease in density is probably responsible for some of the decreases in tensile breaking length, Scott bond strength and modulus. The additional lignin present in the kappa 50 pulp compared to the kappa 23 pulp also appears to contribute to the decrease in strength. Increased mediator contamination or incorporation into the lignin matrix may make some of the modified lignin difficult to remove in an alkaline extraction stage. There was a decrease in pulp physical properties despite an increase in bulk acid group content for some of the samples. However, there was a decrease in handsheet strength properties for the laccase only treated pulp, indicating that laccase alone can produce this decrease in strength properties

All of the treated pulps in this chapter showed a decrease in handsheet strength properties. This result is different from some of the data presented in Chapter 2. In Chapter 2, kappa 23 pulp was treated with laccase and various mediators and not subjected to an alkaline-extracted stage. The Lnhaa treatments for kappa 23 and kappa 50 softwood kraft pulps showed similar trends toward a decrease in handsheet strength. However, the kappa 23 pulp in Chapter 2

showed an increase in tensile breaking length for the Lhbt and Lvio treated pulps. The additional lignin in the kappa 50 pulp may have affected the laccase-mediator chemistry for HBT and VIO. In addition to an increase in the amount of oxidized lignin remaining with the fiber, there may also be a change in the polymerization and deposition of lignin or extractives on the fiber surface. Increased mediator contamination or incorporation into the lignin matrix may also play a role.

CHAPTER 4

PHYSICAL PROPERTIES OF KAPPA 50 PULP TREATED WITH DUPLICATE OXYGEN AND LACCASE-VIOLURIC ACID DELIGNIFICATION

INTRODUCTION

A recent trend in delignification is to install double oxygen stages (107-110) to improve lignin removal, and allow cooking to a higher kappa number. In practice installing a double oxygen stage has only increased the efficiency of an oxygen stage from 50 to 60% (108,110). One reason for the poor performance in the second oxygen stage is lack of washing between stages. It has been suggested that peroxide be added to the second oxygen stage to help overcome the absence of interstage washing (107). The poor performance of the second oxygen stage is believed to be due to the absence of phenolic groups on the remaining lignin, which are essential to further reactivity towards a second stage oxygen. Acid interstage treatments and peroxide are suggested as means of introducing phenolic groups to enhance second-stage delignification (107). However this remains a limitation of oxygen delignification.

In this chapter the effects of a double laccase stage on a high kappa pulp are investigated. Kappa 50 (SKP50-2) was used as the starting material, and violuric acid was chosen as a mediator because of its superior delignification properties. The pulp was treated with single (Lvio-E) and double (Lvio-E-Lvio-E) laccase-violuric acid delignification stages. These results were compared with single and double oxygen delignification stages.

RESULTS AND DISCUSSION

PULP PROPERTIES

Pulp properties of kappa 50 (SKP50-2) pulps treated with single and duplicate Lvio-E and O_2 delignification treatments are shown in Table 24. Laccase-violuric acid was chosen because it is the best laccase-mediator combination for delignification. The starting kappa number of the brownstock starting material was 50.7. Treating the pulp once with Lvio-E brought the kappa number down to 39.5, a decrease of 11.2 points, or 22% delignification. Treatment with an additional Lvio-E delignification resulted in a further decrease in kappa number down to 27.5. This is a 12 point kappa drop and 30% decrease in lignin content. The laccase violuric acid combination increased in efficiency in the second stage.

Oxygen delignification was more effective at removing lignin initially, with the first oxygen delignification resulting in a kappa drop of 17.5, down to 33.2. This is a 35% delignification. The second oxygen delignification brought the kappa number down to 22.8. A 10.4-point drop, or a 31% decrease in lignin content. The decrease in the effectiveness of the second oxygen stage is not as marked as that described in the literature. One reason for this may be that the pulp was thoroughly washed between stages. Another possibility is that the kappa 50 pulp used in this chapter contains more lignin than the starting materials used in the literature sources (~ 25-30).

Laccase violuric acid delignification appears to remove about the same amount of lignin at every treatment (11 or 12 kappa points in this case), and does not lose effectiveness after the first treatment. This suggests that laccase-violuric acid treatment is limited by the amount of

laccase-mediator chemicals present to react, and not by the amount or structure of lignin present in the pulp.

Despite the decrease in kappa number from 50.7 down to 27.5, there was no significant drop in pulp viscosity for the Lvio-E and Lvio-E-Lvio-E treated pulp. The oxygen delignified pulp had a decrease in pulp viscosity from 41.2cp to 24.7cp, and finally to 18.8cp. This is over 50% decrease in pulp viscosity. These data reinforce the fact that the laccase-violuric acid treatment is more selective than oxygen with respect to lignin.

Initial ESCA surface analysis of carboxylic acid groups was 0.71%. This increased to 0.94% for the Lvio-E treated pulp, and then fell again to 0.7% for the Lvio-E-Lvio-E treated pulp. The increase in carboxylic acid groups was also seen for kappa 23 pulp treated with Lvio-E (Chapter 1). The reason for the subsequent decrease in carboxylic acid groups after the second Lvio-E treatment is not known. There was a trend toward a decrease in carboxylic acid content for the O treated pulp, and a trend toward an increase in carboxylic acid content for the OO treated pulp.

Initial bulk acid group content was 0.080meq/g. The bulk acid group content decreased to 0.060meq/g for the Lvio-E pulp. This is probably due to lignin removal. The bulk carboxylic acid group content was unchanged for the Lvio-E-Lvio-E pulp despite lignin removal. It is possible that the second laccase-violuric acid treatment could have oxidized lignin that is more difficult to remove from the fiber in an alkaline extraction stage. There was a significant increase in bulk acid content for the oxygen treated pulp from 0.080 to 0.092meq/g.

Initial acid insoluble lignin was 8.0%. This fell to 6.2% and 4.7% with laccase-violuric acid treatments, and to 5.4% and 4.5% with oxygen treatments. The acid insoluble lignin numbers are in good agreement with the kappa numbers.

Initial ISO brightness was 15.2. The Lvio-E treatment caused an initial decrease in ISO brightness to 11.5. This could be due to mediator conversion, which causes a visual darkening of the liquor and pulp after laccase-violuric acid treatment. The second Lvio-E treatment brought the ISO brightness back up to 14.3. Oxygen delignification caused an increase in ISO brightness to 17.1 and 19.9.

Zero span tensile was initially 100.62Nm/g. There was no significant difference in zero span tensile index for any of the samples. However, there was a trend toward a decrease in zero span for the oxygen treated pulp, and this trend correlates to the drop in pulp viscosity seen in the oxygen treated pulp. The oxygen delignification degrades the carbohydrate portion of the fibers, while the laccase-mediator delignification system does not.

Table 24: Pulp properties of kappa 50 (SKP50-2) pulp treated with duplicate laccase-violuric acid and oxygen treatments.

Sample	Kappa #	Viscosity (mPa/s)	ESCA COOH (%)	Bulk Acid Content (meq/g)	Acid Insoluble Lignin (%)	Bright-ness	Zero Span Tensile (Nm/g)
Standard deviation	±0.3	±0.6	±0.08	±0.006	±0.02	±0.5	±9.5
Untreated	50.7	41.1	0.69	0.082	8.00	15.3	101.6
Lvio-E	39.5	40.4	0.94	0.060	6.20	11.5	98.7
Lvio-E-Lvio-E	27.5	39.4	0.70	0.087	4.70	14.3	110.9
O2	33.2	24.7	0.59	0.094	5.40	17.1	93.2
O2O2	22.8	18.8	0.83	0.092	4.50	19.9	92.9

Table 25 contains the carbohydrate analysis data for the kappa 50 (SKP50-2) pulps treated with single and repeated Lvio-E and O_2 delignification. No significant difference in carbohydrate concentration in the pulp samples was detected. Almost all of the reaction appears

to be isolated in the lignin fraction of the pulp. The high amount of lignin in this pulp may have protected the carbohydrates from removal by reacting first.

Table 25: Carbohydrate analysis of kappa 50 (SKP50-2) pulp treated with duplicate laccase and oxygen treatments. (Standard deviation ± 0.5 for xylan and mannan.)

	Glucan %	Xylan %	Galactan %	Arabinan %	Mannan %
Untreated	70.0	4.6	Nd	0.6	6.7
Lvio-E	73.0	4.5	Nd	0.3	6.7
Lvio-E-Lvio-E	74.3	5.0	Nd	0.2	6.5
O2	73.8	4.9	Nd	0.3	6.7
O2O2	69.2	5.4	Nd	0.8	6.6

The nitrogen content of these pulps is shown in Table 26. There was an increase in nitrogen content from <0.01 to 0.15% for the single Lvio-E treated pulp. A further increase to 0.18% was seen for the Lvio-E-Lvio-E treated pulp. There was no increase for the oxygen treated pulp. The increase in nitrogen content for the laccase violuric acid treated pulp survives the alkaline extraction stage. This indicated that the nitrogen is closely associated, and possibly bonded to the residual lignin in the pulp.

Table 26: Nitrogen content of kappa 50 (SKP50-1) pulps treated with double oxygen and double laccase violuric acid systems.

Sample	% Nitrogen
Standard deviation	± <0.01
Untreated	<0.01
Lvio-E	0.15
Lvio-E-Lvio-E	0.18
O2	0.01
O2O2	0.01

PAPER PHYSICAL PROPERTIES

Physical properties of kappa 50 (SKP50-2) pulp treated with single and duplicate Lvio-E and O_2 delignification are shown in the following 8 figures. The figures are divided into sections according to Lvio-E delignification and oxygen delignification. In each section, the order of paper physical properties is Scott bond strength, tensile breaking length, MOE, and density. P-values are given for changes in slope and intercept. A P-value of 0.05 or less indicates a significant difference.

Lvio-E and Lvio-E-Lvio-E

Figures 61-64 show the physical properties of Lvio-E and Lvio-E-Lvio-E delignified pulps. There is no statistically significant difference in Scott bond strength for the Lvio-E or the Lvio-E-Lvio-E treated pulps (Figure 61). However, there was an increase in tensile breaking length for the Lvio-E treated pulp, but not for the Lvio-E-Lvio-E treated pulp (Figure 62). MOE (Figure 63) and density (Figure 64) were unchanged. A statistical analysis of covariance was done between the Lvio-E and Lvio-E-Lvio-E pulps to investigate the lack of increase in tensile breaking length for the Lvio-E-Lvio-E pulp. The P-values were 0.20 for the slope and 0.28 for the intercept, indicating no statistically significant difference in the tensile breaking length of the two treated pulps. It is likely, in light of these data, that both pulps had an increase in tensile breaking length. The reason for this increase is not clear, due to the lack of increase in Scott bond strength or MOE.

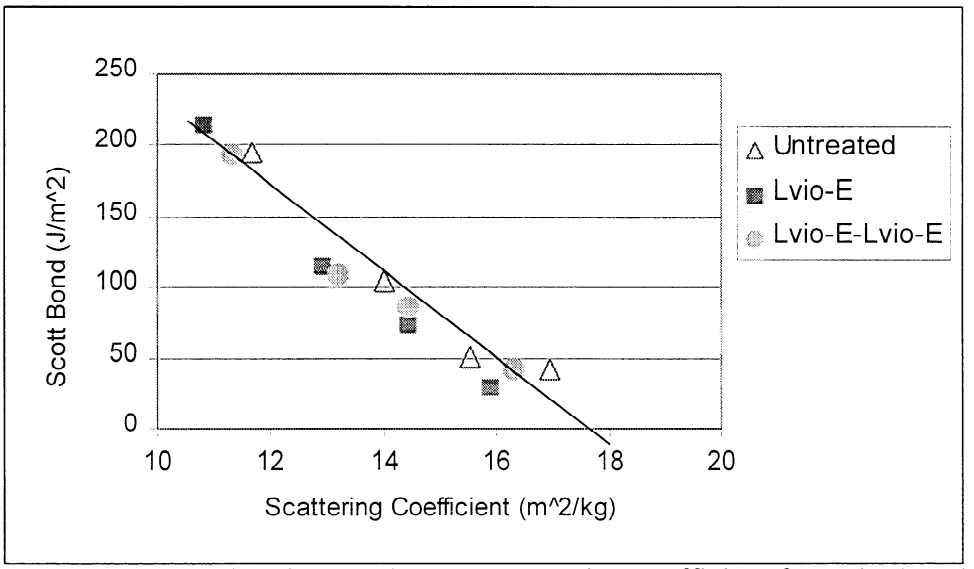

Figure 61: Scott bond strength versus scattering coefficient for Lvio-E and Lvio-E-Lvio-E treated kappa 50 (SKP50-2) pulp. There is no change in slope or intercept. Lvio-E: P-value for differences in slope = 0.41, P-value for differences in intercept = 0.11.Lvio-E-Lvio-E: P-value for differences in slope = 0.93, P-value for differences in intercept = 0.36.

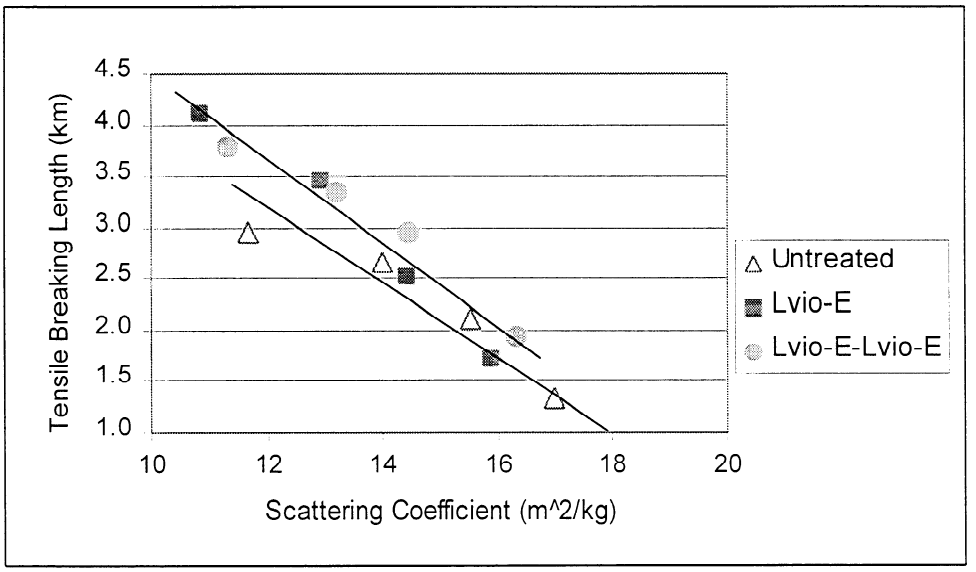

Figure 62: Tensile-breaking length versus scattering coefficient for Lvio-E and Lvio-E-Lvio-E treated kappa 50 (SKP50-2) pulp. There is an increase in intercept for the Lvio-E treated pulp. Lvio-E: P-value for differences in slope = 0.49, P-value for differences in intercept = 0.028. Lvio-E-Lvio-E: P-value for differences in slope = 0.11, P-value for differences in intercept = 0.25. The upper line is for the Lvio-E and Lvio-E-Lvio-E treated pulp, and the lower line is for the untreated pulp.

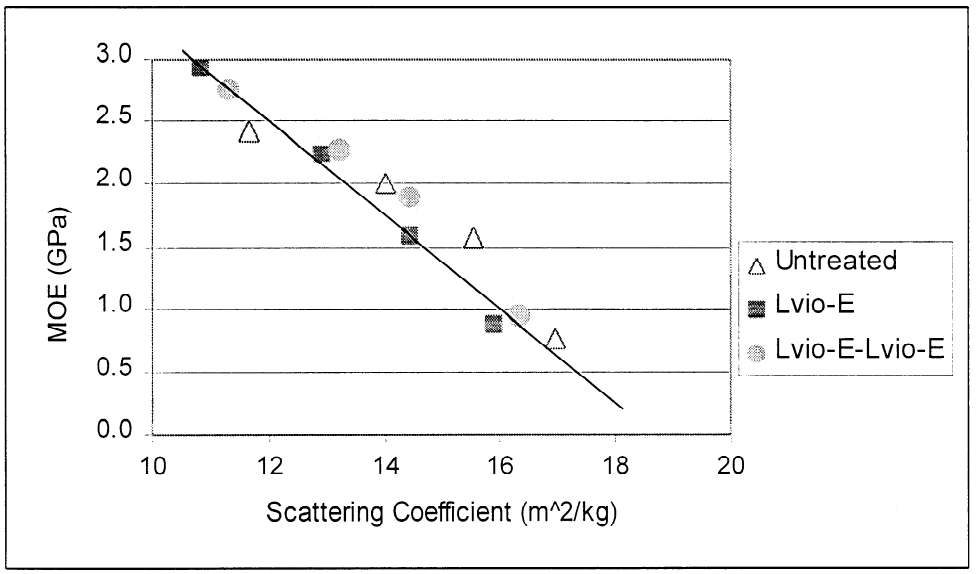

Figure 63: MOE versus scattering coefficient for Lvio-E and Lvio-E-Lvio-E treated kappa 50 (SKP50-2) pulp. There is no change in intercept or slope. Lvio-E: P-value for differences in slope = 0.45, P-value for differences in intercept = 0.79. Lvio-E-Lvio-E: P-value for differences in slope = 0.18, P-value for differences in intercept = 0.38.

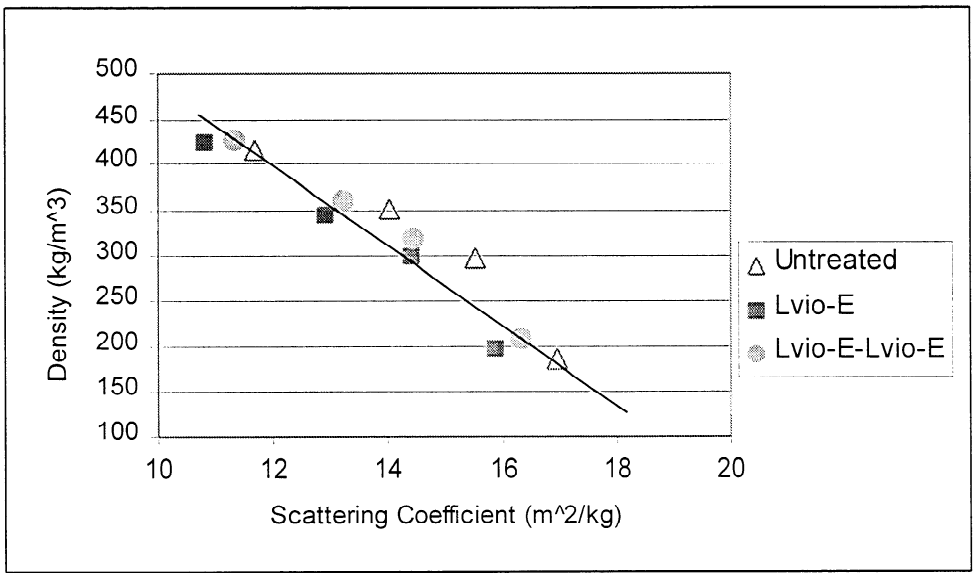

Figure 64: Density versus scattering coefficient for Lvio-E and Lvio-E-Lvio-E treated kappa 50 (SKP50-2) pulp. There is no change in intercept or slope. Lvio-E: P-value for differences in slope = 0.88, P-value for differences in intercept = 0.38. Lvio-E-Lvio-E: P-value for differences in slope = 0.90, P-value for differences in intercept = 0.065.

O2 and O2-O2

Figures 65-68 show the paper physical properties for the single and duplicate oxygen delignified pulps. There was an increase in Scott bond strength for the O_2-O_2 treated pulp, but not for the O_2 treated pulp (Figure 65). Both the O_2 and the O_2-O_2 treated pulps showed an increase in tensile breaking length (Figure 66). There was no change in MOE (Figure 67) or density (Figure 68) for either of these pulps. There was no increase in carboxylic acid content for these pulps. The source of the increase in Scott bond strength for the O_2-O_2 treated pulp could be attributed to lower lignin levels. The O_2-O_2 treated pulp had a significantly lower lignin concentration that the O_2 treated kappa 50 pulp. This could mean less lignin on the surface of the fiber, and greater bonding. This result is in agreement with Laine (26), who found that oxygen delignification removed carboxylic acid rich lignin materials from the surface of kraft pulp fibers.

Lvio-E-Lvio-E versus O2-O2

A comparison was done between the tensile breaking length of the Lvio-E-Lvio-E treated pulp, and the O_2-O_2 treated pulp. The P-values were 0.086 for the slope and 0.41 for the intercept. There is no statistically significant difference between these data, and it must be concluded that the increase in tensile breaking length is the same for these treatments. The higher lignin concentration in the kappa 50 pulps could have helped to protect the fiber from severe degradation of the pulp strength, which can occur by oxygen and especially double oxygen treatment (110).

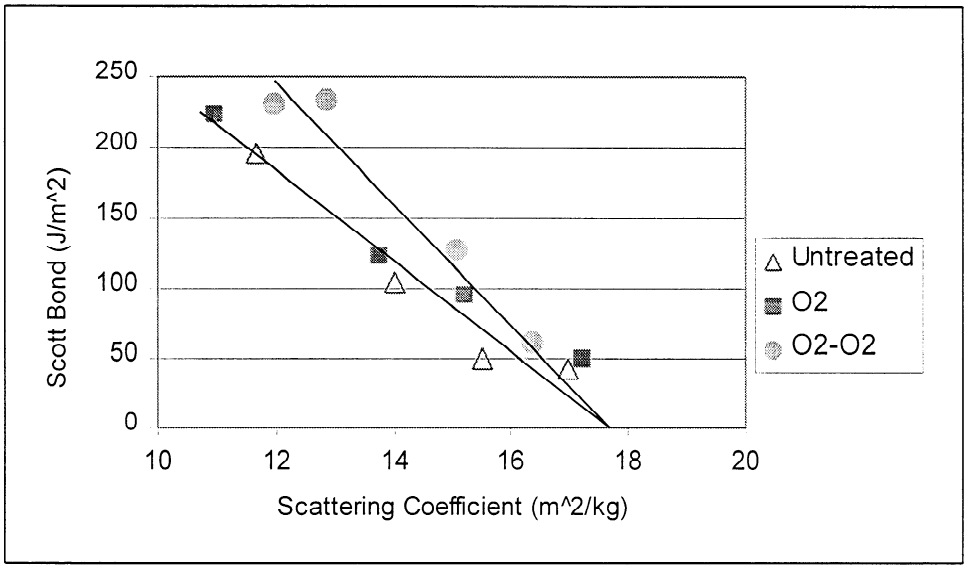

Figure 65: Scott bond strength versus scattering coefficient for O_2 and O_2-O_2 treated kappa 50 (SKP50-2) pulp. There is an increase in intercept for the O_2-O_2 treated pulp. O_2: P-value for differences in slope = 0.69, P-value for differences in intercept = 0.19. O_2-O_2: P-value for differences in slope = 0.24, P-value for differences in intercept = 0.027. The upper line is for the O_2-O_2 treated pulp, and the lower line is for the O_2 and untreated pulps.

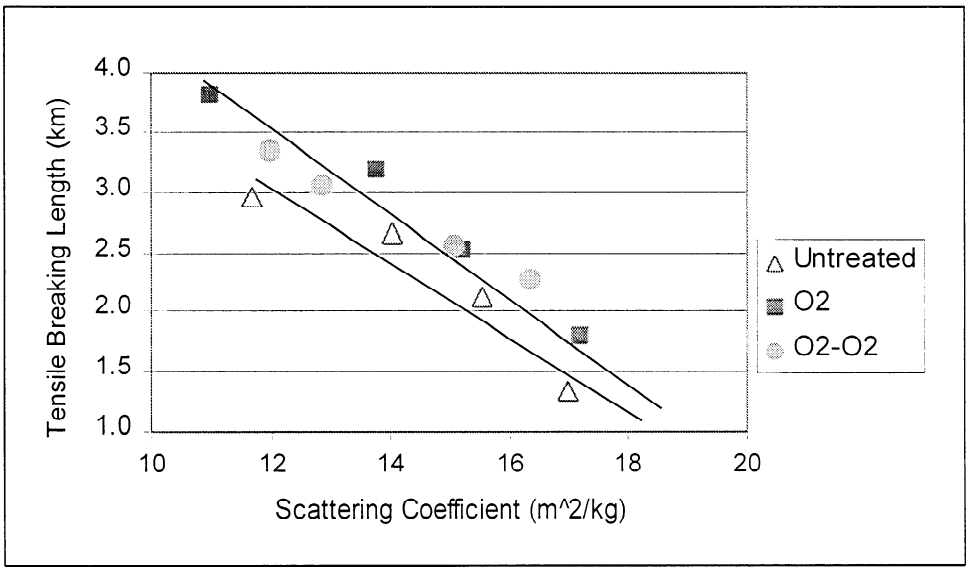

Figure 66: Tensile breaking length versus scattering coefficient for O_2 and O_2-O_2 treated kappa 50 (SKP50-2) pulp. There is an increase in intercept for both the O_2 and the O_2-O_2 treated pulp. O_2: P-value for differences in slope = 0.75, P-value for differences in intercept = 0.020. O_2-O_2: P-value for differences in slope = 0.47, P-value for differences in intercept = 0.027. The upper line is for the O_2 and O_2-O_2 treated pulps, and the lower line is for the untreated pulp.

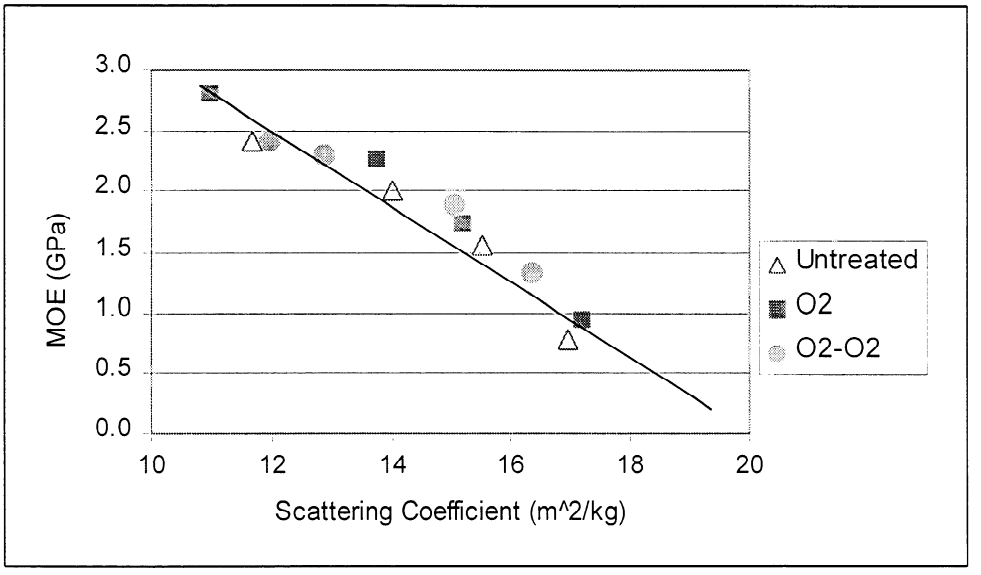

Figure 67: MOE versus scattering coefficient for O_2 and O_2-O_2 treated kappa 50 (SKP50-2) pulp. There is no change in intercept or slope. O_2: P-value for differences in slope = 0.96, P-value for differences in intercept = 0.25. O_2-O_2: P-value for differences in slope = 0.45, P-value for differences in intercept = 0.28.

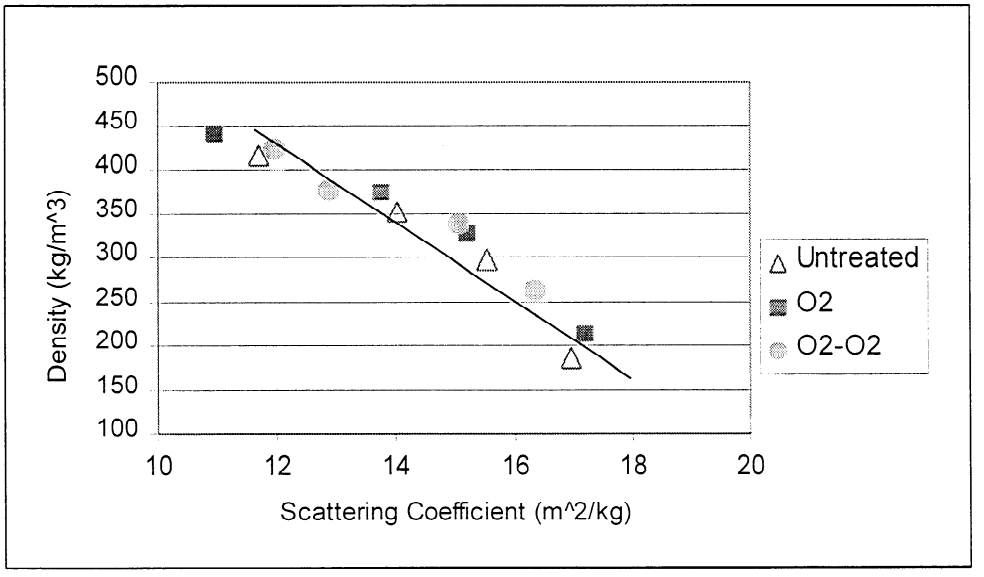

Figure 68: Density versus scattering coefficient for O_2 and O_2-O_2 treated kappa 50 (SKP50-2) pulp. There is no change in intercept or slope. O_2: P-value for differences in slope = 0.55, P-value for differences in intercept = 0.45. O_2-O_2: P-value for differences in slope = 0.40, P-value for differences in intercept = 0.34.

CONCLUSIONS

Consecutive laccase-violuric acid delignification treatments appear to remove about twelve kappa points during each treatment. For kappa 50 pulps, the drop in kappa number does not depend on the amount of lignin present in the pulp. Instead, the efficiency of the laccase-violuric acid delignification system increased, and a greater percentage of lignin was removed during the second stage. This suggests that the residual lignin does not become harder to remove after the first Lvio treatment. This is not the case with oxygen delignification. Oxygen delignification reacts primarily with free phenolic groups, and depletes these groups (107,108). The second oxygen stage is less effective due to the lack of uncondensed phenolic groups. Laccase-mediator delignification does not deplete the free phenolic group content (3), and does not lose effectiveness after the first treatment.

There was only a very small drop in pulp viscosity for the repeated laccase-violuric acid treated pulp, as opposed to the decrease of over 50% for the oxygen delignified pulp. This is a further indication that the laccase-violuric acid system is selective with respect to lignin. However, the pulp viscosity drop due to oxygen delignification did not translate into lower strength properties.

Both laccase-violuric acid and oxygen delignification increase the tensile breaking length of the pulp. However, there was no statistically significant difference between the tensile breaking length of the Lvio-E-Lvio-E and the O_2-O_2 treated pulps, and both treatments appear to increase the tensile strength of the pulp to the same degree.

CHAPTER 5

PHYSICAL PROPERTIES OF KAPPA 50 PULP TREATED WITH LACCASE-VIOLURIC ACID FOLLOWING ACETONE EXTRACTION

INTRODUCTION

Hassingbow et al. suggested that a naturally occurring mediator capable of reacting with laccase might be present in wood extractives (100). In an effort to find this mediator, he extracted mechanical pulp fibers with a variety of solvents, including water and acetone, and concentrated the extracted material. He used these concentrated extracted material in a series of laccase treatments on pulp, and measured and compared oxygen consumption (100). He found a slight increase in oxygen consumption during laccase treatment of TMP fibers which had been previously extracted with acetone, over the oxygen consumption of fibers which had been previously extracted with water. Based on thee experiments he suggested that naturally occurring water soluble mediators might be present in TMP fibers. He did not investigate the effects of removing wood extractives on delignification. This research introduces the possibility that laccase might react with naturally occurring wood extractives.

Both wood extractives and additives such as defoamers have been shown to effect bonding (26,63). Processes such as bleaching and washing can allow a thin layer of extractives to coat the surface of kraft pulp fibers (63). In this chapter, kappa 50 (SKP50-2) pulp was acetone extracted, and then treated with Lvio and Lvio-E. The effects of acetone extraction on delignification of laccase-violuric acid and handsheet physical properties are examined. The removal of wood extractive materials prior to laccase treatment prevents their participation in the

laccase-mediator reaction. When extractives are removed, they are not present to coat the surface of the fibers during laccase-mediator treatment. In chapter 3 there were negative changes in handsheet physical properties following laccase-mediator delignification. These changes may have been due to the polymerization or coating of extracted material onto the surface of the fibers. If this is the case, then the treatment of acetone extracted pulp in this chapter should not produce a decrease in handsheet physical properties.

RESULTS AND DISCUSSION

PULP PROPERTIES

Pulp properties for kappa 50 (SKP50-2) pulps soxhlet extracted with acetone and then treated with laccase-violuric acid are shown in Table 27. The acetone extraction step removed much of the wood extractives, leaving the cellulose, hemicellulose, and lignin behind. This allowed us to see what effect wood extractives had on the laccase-violuric acid delignification reaction. The starting kappa number of the acetone-extracted pulp was 47.4. The kappa number fell to 41.4 after a Lvio treatment, and to 36.5 after a Lvio-E sequence. The kappa drop of 10.9 points is close to the kappa drop of 11 or 12 for the Lvio-E delignified kappa 50 pulp in Chapter 4, which was not acetone extracted. The presence or absence of extractives does not seem to affect the effectiveness of the Lvio-E delignification chemistry.

The pulp viscosity of the acetone-extracted starting material was 43.0cp, slightly above the 41.2cp of the untreated control pulp. The pulp viscosity decreased to 39.0cp and 38.6cp after treatment with Lvio and Lvio-E. This is a larger pulp viscosity drop than was seen for the Lvio-E

(viscosity 40.4) treatment in Chapter 4. The removal of extractives may have an effect on the selectivity of the laccase-violuric acid chemistry.

The surface carboxylic acid value of the acetone-extracted starting material was 0.56%. ESCA measurement of surface carboxylic acid groups increased to 1.25% for the Lvio treatment, and went back down to 0.74% for the Lvio-E treatment. The absence of wood extractives appears to enhance the ability of the Lvio chemistry to introduce acid groups in lignin. The decrease in surface carboxylic acid content from the Lvio to the Lvio-E pulp indicates that the acid groups on the surface of the fiber are present in lignin. The highly oxidized lignin fragments were removed during the alkaline extraction stage.

Bulk acid group measurements showed quite different results. The bulk acid group content of the acetone-extracted starting material was 0.097meq/g. This value fell to 0.050meq/g for the Lvio treatment, and then increased again to 0.123meq/g for the Lvio-E treated pulp. One possible explanation for this is orthoquinone structures that rearrange during alkaline extraction stage to form furane carboxylic acid structures (19). This suggests that a significant number of quinone structures are generated during the laccase-violuric acid treatment. This is consistent with other research (3, 111-113). Lundquist (113) reacted syringic acid (model compound) with laccase and found up to 18% formation of orthoquinone structures. The mechanism of formation of quinone structures by laccase is shown in Figure 69 (113).

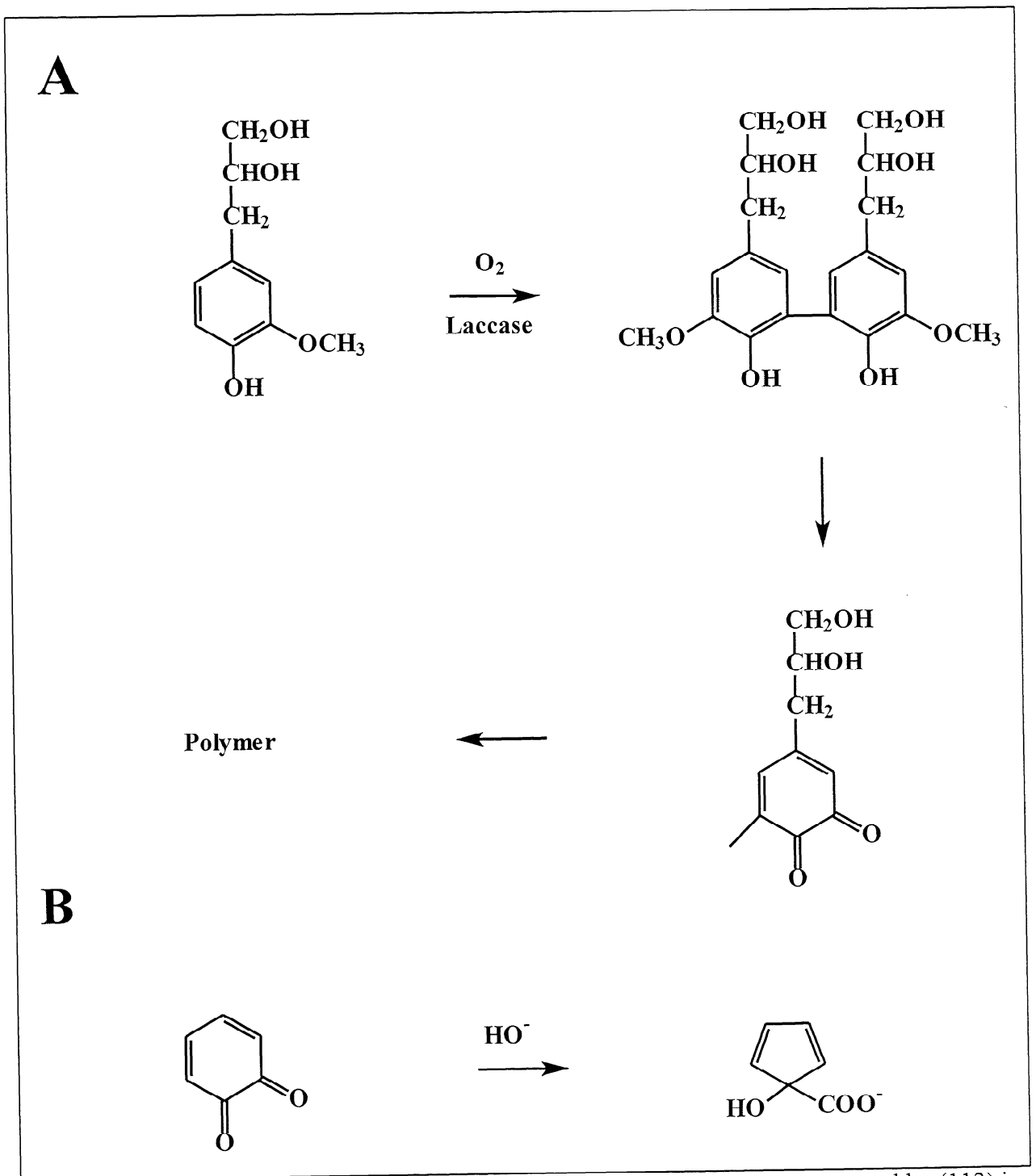

Figure 69: Formation of quinone structures during laccase treatment. As represented by (113) in pathway A, and (19) in pathway B.

The acid insoluble lignin represented 7.6% of the acetone-extracted starting material. Acid insoluble lignin decreased to 6.6% for the Lvio treated pulp, and again to 5.8% for the Lvio-E treated pulp. These data are in agreement with the kappa number results.

Initial ISO brightness of the acetone extracted starting material was 15.9. This value fell to 9.9 for the Lvio treated pulp, and went up again to 11.2 following alkaline extraction (Lvio-E). The decrease in ISO brightness is due to alkali darkening, and mediator contamination. There was no significant difference in zero span tensile data, indicating no decrease in fiber strength.

Table 27: Pulp properties of kappa 50 (SKP50-2) acetone-extracted pulp treated with laccase-violuric acid.

Sample	Kappa #	Viscosity (mPa/s)	ESCA COOH (%)	Bulk Acid Content (meq/g)	Acid Insoluble Lignin (%)	Bright -ness	Zero Span Tensile (Nm/g)
Standard deviation	±0.3	±0.6	±0.08	±0.006	±0.02	±0.5	±9.5
Untreated	50.7	41.1	0.69	0.082	8.00	15.3	101.6
A-Untreated	47.4	43.0	0.56	0.097	7.60	15.9	102.8
A-Lvio	41.4	39.0	1.25	0.050	6.60	9.9	101.7
A-Lvio-E	36.5	38.6	0.74	0.123	5.80	11.2	106.7

Table 28 contains the carbohydrate analysis data for the kappa 50 (SKP50-2) acetone-extracted and laccase-violuric acid treated pulps. There are no significant differences in carbohydrate concentration. The reaction appears to be selective with respect to lignin, and does not remove much hemicellulose.

Table 28: Carbohydrate analysis of kappa 50 (SKP50-2) acetone-extracted pulp treated with laccase-violuric acid. (Standard deviation ± 0.5 for xylan and mannan)

	Glucan %	Xylan %	Galactan %	Arabinan %	Mannan %
Untreated	70.0	4.6	nd	0.6	6.7
A-Untreated	73.4	4.9	nd	0.2	6.6
A-Lvio	73.9	5.1	nd	0.4	6.9
A-Lvio-E	74.1	4.8	nd	0.2	6.6

The nitrogen content of these pulps is shown in Table 29. There was an increase in nitrogen content from <0.01 to 0.27% for the Lvio treated pulp. Following alkaline extraction, the nitrogen content fell to 0.14%. This number is in close agreement with the 0.15% nitrogen content for a Lvio-E treated kappa 50 pulp in chapter 4. The absence of wood extractives dose not effect the nitrogen contamination of the pulp.

Table 29: Nitrogen content of kappa 50 (SKP50-1) acetone extracted pulp treated laccase violuric acid.

Sample	% Nitrogen
Standard deviation	± <0.01
Untreated	<0.01
A-Untreated	0.01
A-Lvio	0.27
A-Lvio-E	0.14

PAPER PHYSICAL PROPERTIES

Physical properties of acetone-extracted kappa 50 (SKP50-2) pulps treated laccase-violuric acid delignification are shown in the following 4 figures. The order of test measurement

is Scott bond strength, tensile breaking length, MOE, and density. P-values are given for changes in slope and intercept for each line. A P-value of 0.05 or less indicates a significant difference.

Acetone extracted pulp treated with Laccase-VIO

Figures 70-73 show the physical properties of acetone-extracted and laccase-violuric acid delignified pulps. There was no change in Scott bond strength for any of the treatments (Figure 70). There was an increase in ESCA measurement of surface carboxylic acid groups for the A-Lvio treated pulp, and it is clear that the surface acid groups in lignin do not have an effect on the bond strength of this pulp. There was no change in tensile breaking length (Figure 71), or in MOE (Figure 72) for these pulps. There was a decrease in density for the A-Lvio and A-Lvio-E treated pulps (Figure 73), but this slight change in density had no effect on other paper properties.

The decrease in Scott bond strength associated with the Lvio treatment (Chapter 3) was not seen for the A-Lvio treated pulp in this chapter. The presence of wood extractives in the pulp appears to have been responsible for the decrease in strength seen in the kappa 50 Lvio treated pulp in Chapter 3. The Lvio-E pulps in this chapter and in Chapter 4, did not show a change in Scott bond strength. The alkaline extraction stage appears to undo the damage done by wood extractives in the Lvio treatment in Chapter 3.

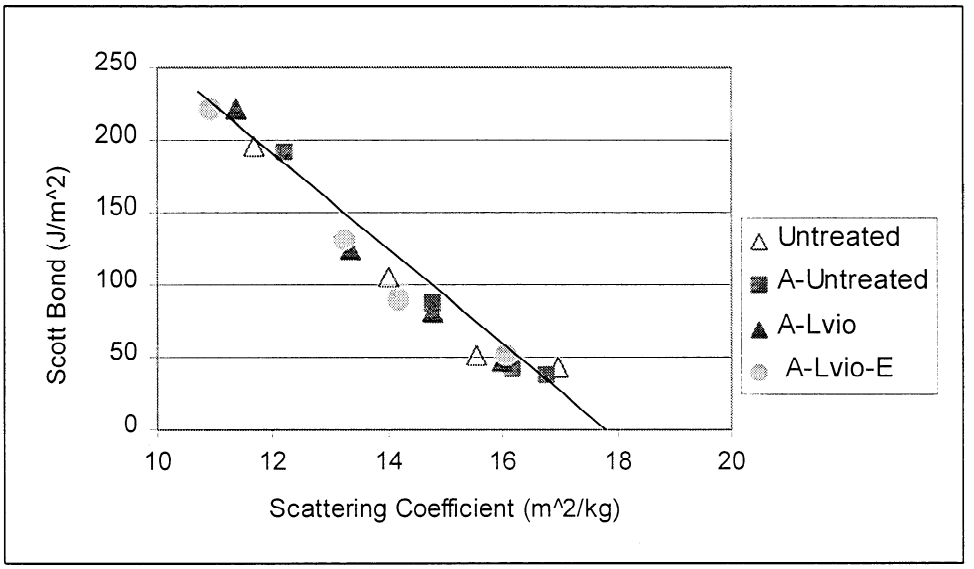

Figure 70: Scott bond strength versus scattering coefficient for acetone extracted kappa 50 (SKP50-2) pulp treated with laccase-violuric acid. There was no change in slope or intercept for any of the treatments. A-Untreated: P-value for differences in slope = 0.47, P-value for differences in intercept = 0.66. A-Lvio against A-Untreated: P-value for differences in slope = 0.69, P-value for differences in intercept = 0.23. A-Lvio-E against A-Untreated: P-value for differences in slope = 0.72, P-value for differences in intercept = 0.18.

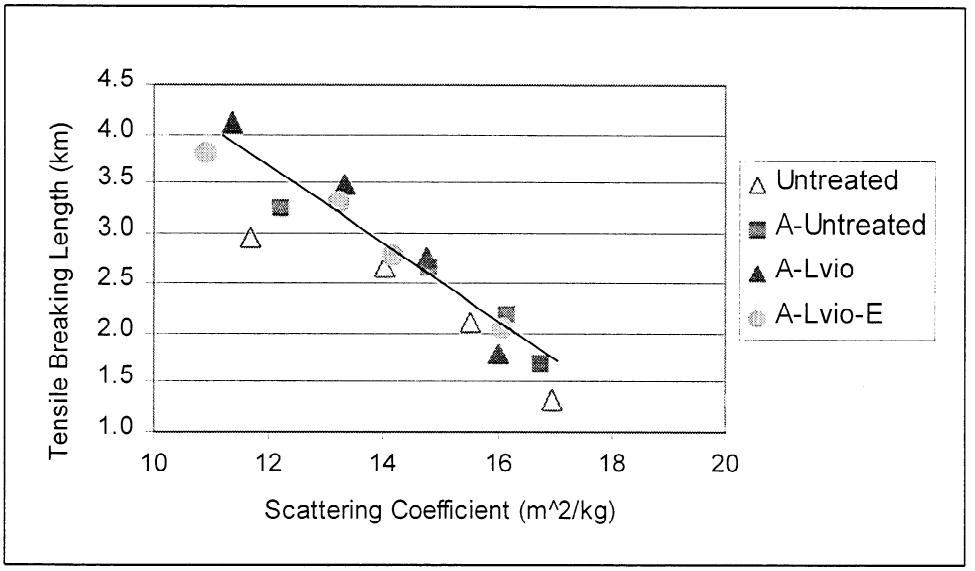

Figure 71: Tensile breaking length versus scattering coefficient for acetone extracted kappa 50 (SKP50-2) pulp treated with laccase-violuric acid. There was no change in intercept or slope for the Lvio or Lvio-E treated pulps. A-Untreated: P-value for differences in slope = 0.87, P-value for differences in intercept = 0.097. A-Lvio against A-Untreated: P-value for differences in slope = 0.13, P-value for differences in intercept = 0.50. A-Lvio-E against A-Untreated: P-value for differences in slope = 0.69, P-value for differences in intercept = 0.50.

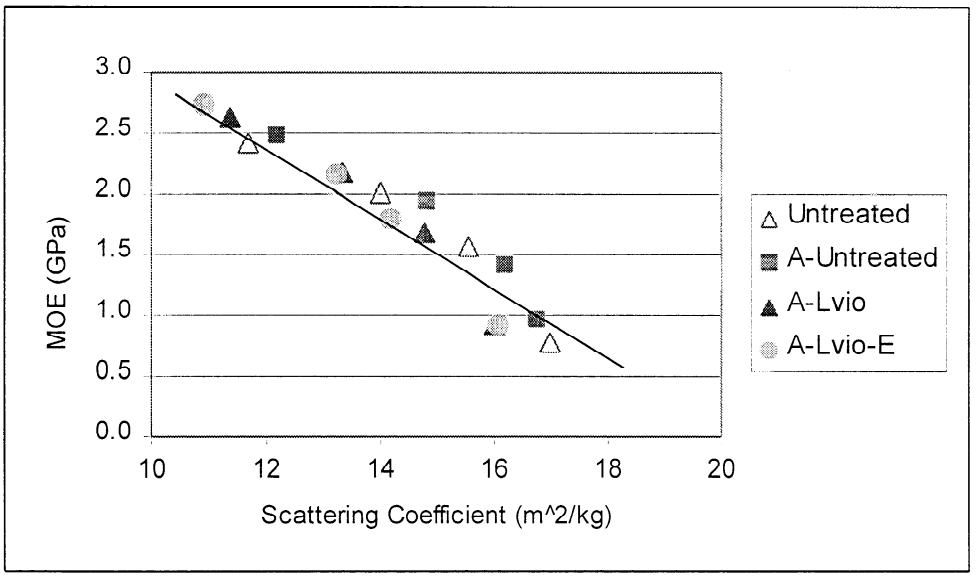

Figure 72: MOE versus scattering coefficient for acetone extracted kappa 50 (SKP50-2) pulp treated with laccase-violuric acid. There was no change in slope or intercept for any of the treatments. A-Untreated: P-value for differences in slope = 0.85, P-value for differences in intercept = 0.34. A-Lvio against A-Untreated: P-value for differences in slope = 0.62, P-value for differences in intercept = 0.16. A-Lvio-E against A-Untreated: P-value for differences in slope = 0.69, P-value for differences in intercept = 0.092.

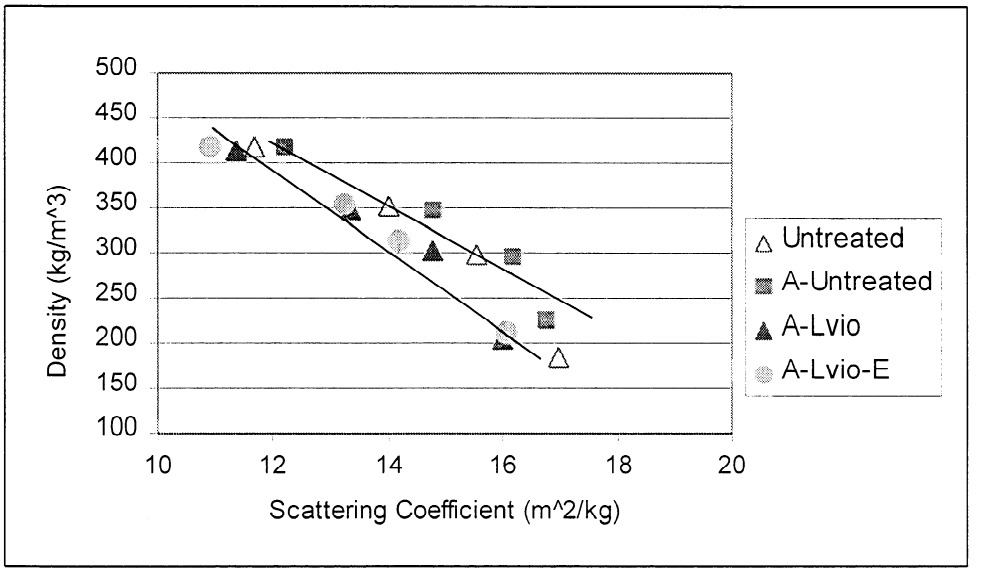

Figure 73: Density versus scattering coefficient for acetone extracted kappa 50 (SKP50-2) pulp treated with laccase-violuric acid. There is a decrease in density for the A-Lvio and the A-Lvio-E treated pulps. A-Untreated: P-value for differences in slope = 0.79, P-value for differences in intercept = 0.24. A-Lvio against A-Untreated: P-value for differences in slope = 0.74, P-value for differences in intercept = 0.039. A-Lvio-E against A-Untreated: P-value for differences in slope = 0.91, P-value for differences in intercept = 0.027. The upper line is for the a-Untreated pulp, and the lower line is for the A-Lvio and A-Lvio-E treated pulps.

CONCLUSIONS

Wood extractives had no effect on the efficiency of the laccase-violuric acid system to remove lignin. Wood extractives do not appear to be involved in the delignification reaction. The handsheet strength properties in this chapter are in agreement with the strength of the Lvio-E treated pulp in Chapter 4. However, the removal of extractives prevented the decrease in Scott bond strength for the Lvio treated pulp. The extractives may have decreased the strength of the pulp by coating the surface of the pulp and reducing bonding. This indicates that the decrease in handsheet physical properties seen in Chapter 3 may have been due to the presence of extractives.

In the absence of wood extractives, the laccase-violuric acid reaction may form more orthoquinones in the lignin material in kappa 50 pulp (111-113). These quinones are most likely oxidized to furane carboxylic acid groups in the alkaline extraction stage. These acid groups in lignin do not appear to have any effect on handsheet physical properties.

There was an increase in ESCA measurement of surface carboxylic acid groups for the A-Lvio treated pulp. The surface carboxylic acid measurement decreased substantially following alkaline extraction, indicating that the majority of the surface acid groups were located in reacted lignin fragments. These acid groups had no effect on Scott bond strength or tensile breaking length. It appears that acid groups in lignin do not contribute to specific bond strength for these pulps.

CHAPTER 6

PHYSICAL PROPERTIES OF LACCASE-MEDIATOR TREATED FULLY BLEACHED PULP

INTRODUCTION

The effects of laccase-mediator systems have been thoroughly studied for kappa 23 and kappa 50 softwood kraft pulps. In Chapter 5 the laccase-violuric acid combination increased the surface carboxylic acid content of lignin without the improvement in specific bond strength. The laccase-mediator systems have been shown to be selective with respect to lignin, and not degrade cellulose or remove hemicellulose. However, the question remains as to whether the laccase-mediator systems might also have an impact on the carbohydrate fraction.

This chapter examines the effects of laccase-mediator treatment on pulp and paper physical properties, and compares them to an oxygen delignification treatment. The fully bleached pulp was obtained from Weyerhaeuser, and was treated with laccase-mediator systems and then alkaline-extracted. These pulps are compared with an oxygen delignification treatment. The control pulp in this study is an alkaline extraction treated pulp.

Carboxylic acid groups can be introduced to fully bleached pulps through oxygen delignification (5,101), and through alkaline extraction (95). The introduction of acid groups during alkaline extraction is believed to be due to the peeling reaction, and is dependent on the formation of organic hydroperoxides which form between 80° and 110°C (94,95). Increases in specific bond strength have been associated with the formation of carboxylic acid groups on the surface of fully bleached pulp during oxygen delignification (5).

RESULTS AND DISCUSSION

PULP PROPERTIES

Pulp properties for fully bleached pulp (FBP) treated with laccase and the various mediators and oxygen are located in Table 30. The pulps were treated with the laccase-mediator chemicals and then alkaline-extracted to remove any oxidized material. The alkaline extraction also removed any deposited enzyme or mediator chemicals.

The pulp viscosity of the alkaline-extracted material was 21.0cp. Treatment with Lhbt-E decreased the pulp viscosity down to 19.9cp. Lnhaa-E treatment lowered the pulp viscosity down to 16.0cp, and Lvio-E treatment decreased the pulp viscosity to 17.5cp. Both O_2 and Lvio-E-Lvio-E treatments decreased the pulp viscosity to 16.4cp.

ESCA measurement of surface acid groups was 0.51% for the alkaline-extracted starting material. There were no significant changes in ESCA data for any of the treatments, indicating no change in surface acid content. Bulk acid content of the alkaline-extracted starting material was 0.032%, and did not change significantly for any of the treatments.

The ISO brightness of the alkaline-extracted material was 79.0. This number increased to 82.1 after an oxygen delignification. Lhbt-E and Lnhaa-E both caused a decrease in ISO brightness to 76.9 and 77.9, respectively. This decrease in brightness is probably due to residual mediator material. The Lvio-E treatment had no effect on ISO brightness, and the Lvio-E-Lvio-E treatment caused a slight increase in ISO brightness to 80.2. The laccase-mediator systems are not very effective at brightening pulp, and can decrease brightness even after an alkaline extraction stage. The residual color after laccase-violuric acid treatment is completely removed from the pulp after an alkaline extraction stage. Laccase-HBT and laccase-NHAA do not

completely recover their brightness values after an alkaline extraction stage. There was no significant change in zero span tensile data indicating no change in fiber strength.

Table 30: Pulp properties of fully bleached pulp (FBP) treated with laccase-mediator systems.

Sample	Viscosity (mPa/s)	ESCA COOH (%)	Bulk Acid Content (meq/g)	Bright-ness	Zero Span Tensile (Nm/g)
Standard deviation	±0.6	±0.08	±0.006	±0.5	±9.5
Untreated	20.9	0.46	0.030	78.1	100.7
E	21.0	0.51	0.032	79.0	96.7
O2	16.4	0.46	0.030	82.1	90.9
Lhbt-E	19.9	0.39	0.032	76.9	99.6
Lnhaa-E	16.0	0.44	0.033	77.9	99.0
Lvio-E	17.5	0.43	0.027	79.0	100.7
Lvio-E-Lvio-E	16.4	0.40	0.027	80.2	94.0

Table 31 shows the carbohydrate analysis data for the fully bleached pulps (FBP) treated with laccase and various mediators and oxygen. The results show no significant removal of hemicellulose materials. This indicates that the laccase-mediator systems are selective with respect to lignin, and do not remove hemicellulose.

Table 31: Carbohydrate analysis of fully bleached pulps (FBP). (Standard deviation + 0.5 for xylan and mannan)

	Glucan %	Xylan %	Galactan %	Arabinan %	Mannan %	Total Carbo-hydrates%
Untreated	79.5	6.6	nd	0.6	6.2	92.9
E	78.6	6.1	nd	0.7	6.3	91.6
O2	79.0	5.9	nd	nd	5.6	90.6
Lhbt-E	77.3	6.3	nd	0.6	6.0	90.2
Lnhaa-E	78.2	6.4	nd	0.7	6.1	91.4
Lvio-E-Lvio-E	77.6	6.6	nd	0.3	6.0	90.5

The nitrogen content and surface extractives content of these pulps is shown Table 32. There was no change in nitrogen content for any of the treatments. This indicates that the nitrogen from both enzyme and/or mediator sources is bound to the lignin fraction of the pulp, and not the cellulose or hemicellulose fractions. This further supports the conclusion that the laccase-mediator systems are selective with respect to lignin.

Surface extractive content was determined from the C 1s-1 carbon orbital peal as measured before and after acetone extraction. Surface extractives content of the starting material was 15.30%. After treating the pulp with E, O_2, Lhbt-E, Lnhaa-E, and Lvio-E stages, the surface extractives content fell to about 10%. The Lvio-E-Lvio-E treated pulp fell to 7.45%. There was a similar decrease in surface extractive content for each of the treated pulps, with the exception of the Lvio-E-Lvio treated pulp, which showed a slightly greater decrease. These pulp treatments appear to clean up the surface of the pulp fibers. The removal of extractives from the fiber surface would allow greater contact between cellulose interfaces. In this way the natural bonding ability of the cellulose should be maximized.

Table 32: Nitrogen analysis and ESCA results of extractives on the paper surface of fully bleached pulp (FBP) treated with laccase-mediator systems.

Sample	% Nitrogen	ESCA (%) extractives on surface
Standard deviation	± <0.01	± 1.40
Untreated	<0.01	15.30
E	<0.01	9.21
O2	<0.01	10.77
Lhbt-E	<0.01	10.16
Lnhaa-E	<0.01	9.34
Lvio-E	<0.01	10.95
Lvio-E-Lvio-E	<0.01	7.45

PAPER PHYSICAL PROPERTIES

The physical properties of fully bleached pulp (FBP) treated with laccase-mediator systems and oxygen are shown in the following 16 figures. They are divided into sections in the following order: Lhbt-E, Lnhaa-E, Lvio-E, and O_2. In each section, the order of test measurement is Scott bond strength, tensile breaking length, MOE, and density. P-values are given for changes in intercept and slope for each line. A P-value of 0.05 or less indicates a significant difference. All comparisons are made between the treated pulps and the alkaline-extracted pulp.

Lhbt-E

Figures 74-77 show the physical properties of Lhbt-E treated pulp. There was no change in Scott bond strength (Figure 74), tensile breaking length (Figure 75), MOE (Figure 76), or density (Figure 77) for this treatment. Treatment with Lhbt-E appears to have no effect on paper physical properties when compared to an alkaline-extracted control pulp. This result is in agreement with the data from Chapter 1 in that there were no observed changes in handsheet strength properties

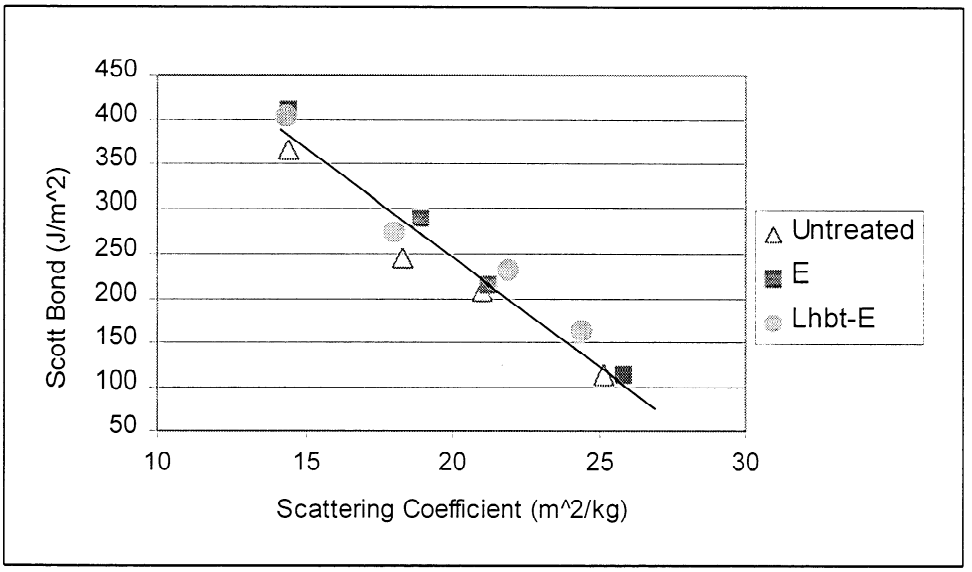

Figure 74: Scott bond strength versus scattering coefficient for Lhbt-E treated fully bleached pulp (FBP). There is no change in slope or intercept. P-value for differences in slope = 0.35, P-value for differences in intercept = 0.097.

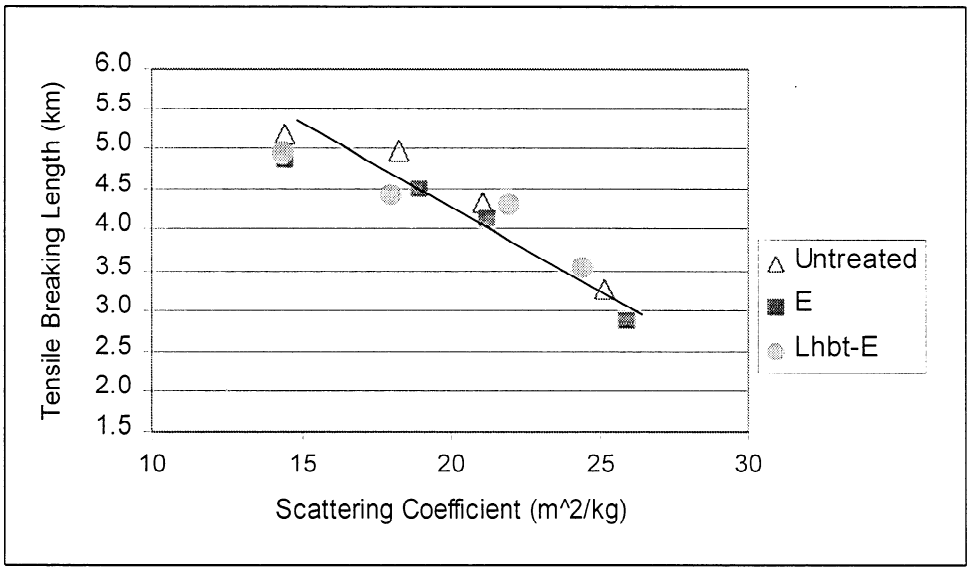

Figure 75: Tensile breaking length versus scattering coefficient for Lhbt-E treated fully bleached pulp (FBP). There is no change in slope or intercept. P-value for differences in slope = 0.35, P-value for differences in intercept = 0.51.

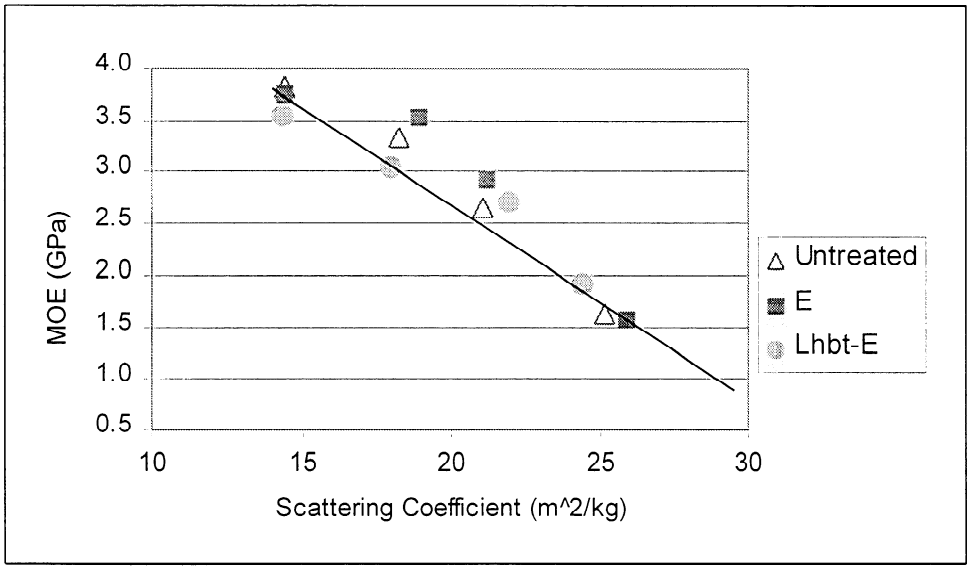

Figure 76: MOE versus scattering coefficient for Lhbt-E treated fully bleached pulp (FBP). There is no change in slope or intercept. P-value for differences in slope = 0.48, P-value for differences in intercept = 0.37.

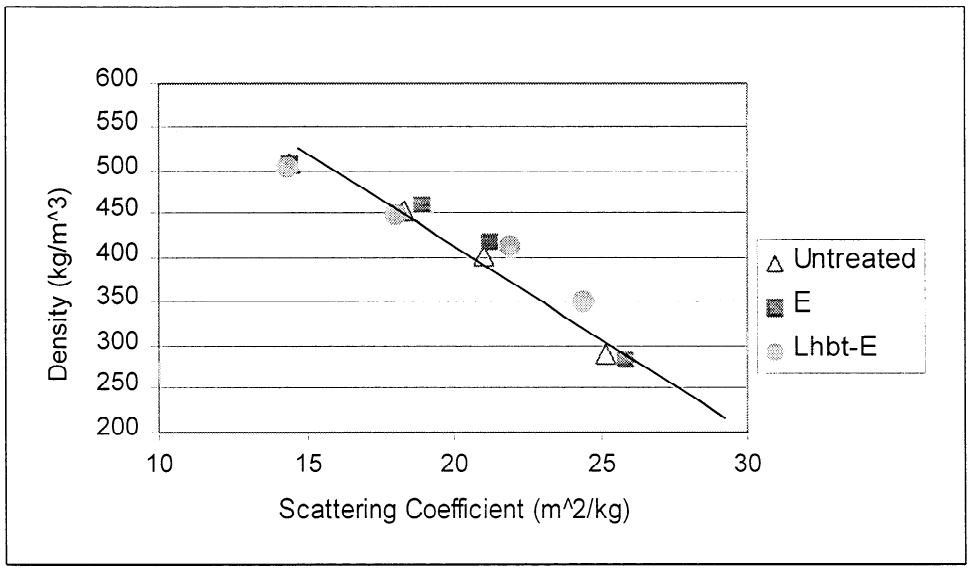

Figure 77: Density versus scattering coefficient for Lhbt-E treated fully bleached pulp (FBP). There is no change in slope or intercept. P-value for differences in slope = 0.27, P-value for differences in intercept = 0.83.

Lnhaa-E

Figures 78-81 show the physical properties of Lnhaa-E treated pulp. There was no change in Scott bond strength (Figure 78), no change in tensile breaking length (Figure 79), no change in MOE (Figure 80), and no change in density (Figure 81). Treatment with Lnhaa-E appears to have no effect on paper physical properties when compared with an alkaline-extracted control pulp. This result agrees with that of Chapter 1 in that there was no change in Scott bond strength or tensile breaking length. In Chapter 1 there was a decrease in MOE for Lnhaa-E treated kappa 23 pulp which was not seen in fully bleached pulp. The lignin reaction with NHAA may have caused the decrease in MOE seen in previous chapters.

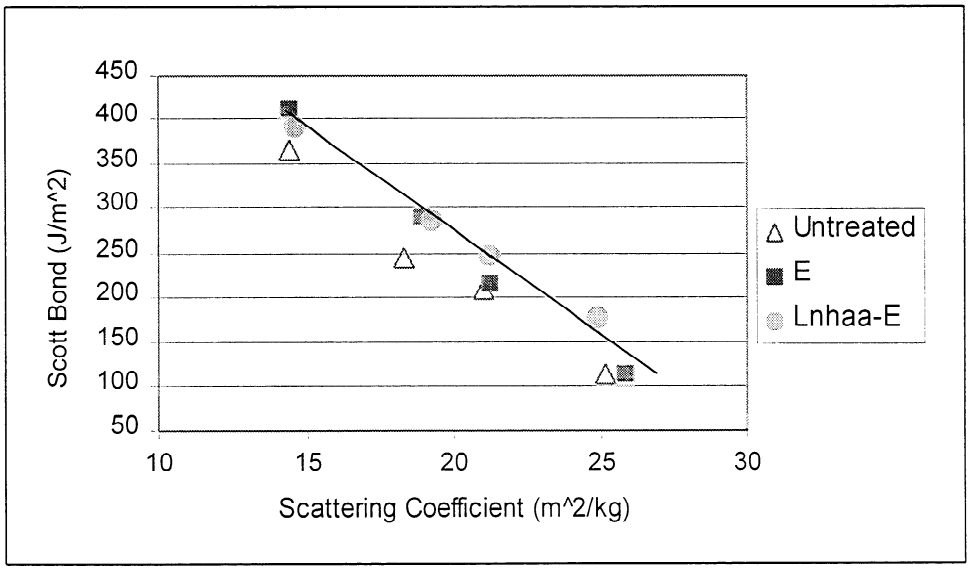

Figure 78: Scott bond strength versus scattering coefficient for Lnhaa-E treated fully bleached pulp (FBP). There is no change in slope or intercept. P-value for differences in slope = 0.042, P-value for differences in intercept = 0.21.

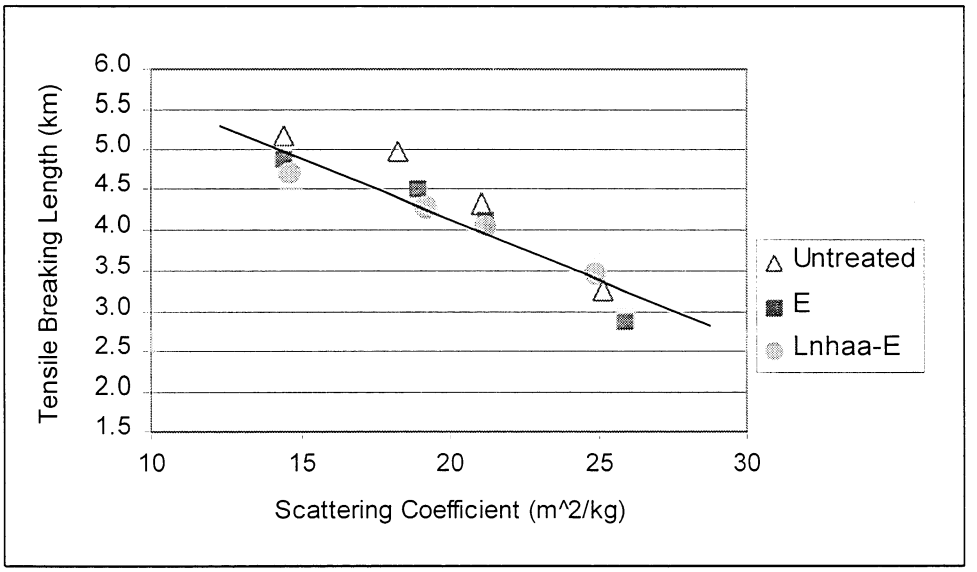

Figure 79: Tensile breaking length versus scattering coefficient for Lnhaa-E treated fully bleached pulp (FBP). There is no change in slope or intercept. P-value for differences in slope = 0.25, P-value for differences in intercept = 0.95.

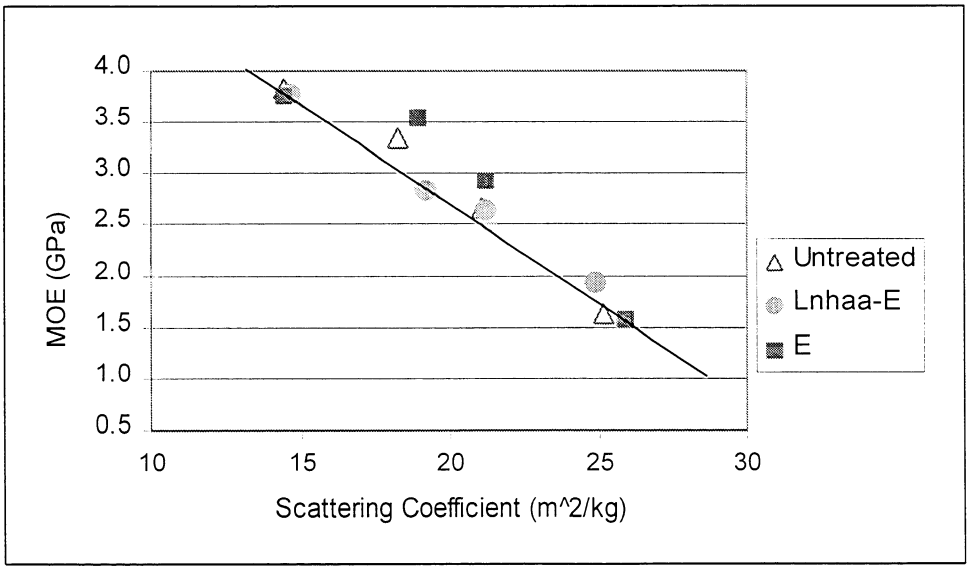

Figure 80: MOE versus scattering coefficient for Lnhaa-E treated fully bleached pulp (FBP). There is no change in slope or intercept. P-value for differences in slope = 0.76, P-value for differences in intercept = 0.41.

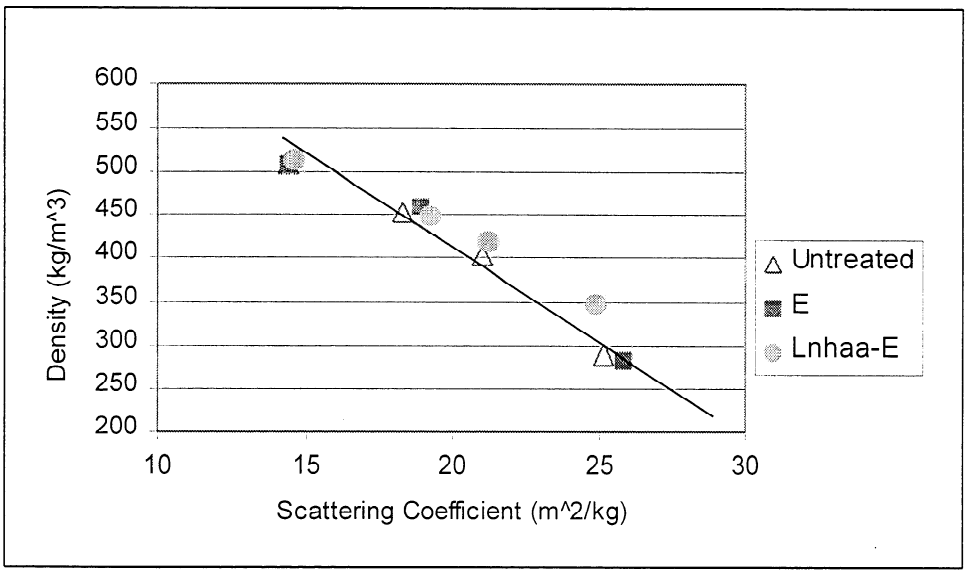

Figure 81: Density versus scattering coefficient for Lnhaa-E treated fully bleached pulp (FBP). There is no change in slope or intercept. P-value for differences in slope = 0.40, P-value for differences in intercept = 0.46.

Lvio-E and Lvio-E-Lvio-E

Figures 82-85 show the physical properties of Lvio-E and Lvio-E-Lvio-E treated pulp. There was no change in Scott bond strength (Figure 82), and no change in tensile breaking length (Figure 83) for the pulps. There was a slight decrease in MOE for the Lvio-E-Lvio-E treated pulp (Figure 84), but this result did not affect tensile breaking length. There was no change in density (Figure 85). Aside from a slight decrease in MOE for the double Lvio-E treated pulp, the laccase-violuric acid treatment had no effect on paper physical properties of fully bleached pulp. This is in contrast to the data in Chapter 1, where Lvio-E treated pulp showed an increase in Scott bond strength and tensile breaking length. The improvement in handsheet strength properties seen in Chapter 1 was most likely due to delignification and removal of lignin.

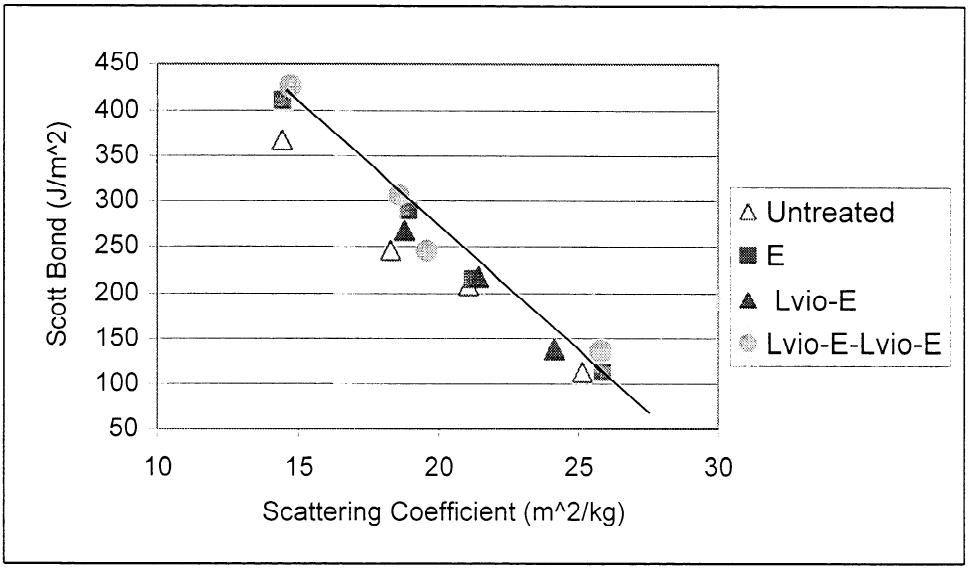

Figure 82: Scott bond strength versus scattering coefficient for Lvio-E and Lvio-E-Lvio-E treated fully bleached pulp (FBP). There is no change in slope or intercept. Lvio-E: P-value for differences in slope = 0.61, P-value for differences in intercept = 0.17. Lvio-E-Lvio-E: P-value for differences in slope = 0.93, P-value for differences in intercept = 0.51.

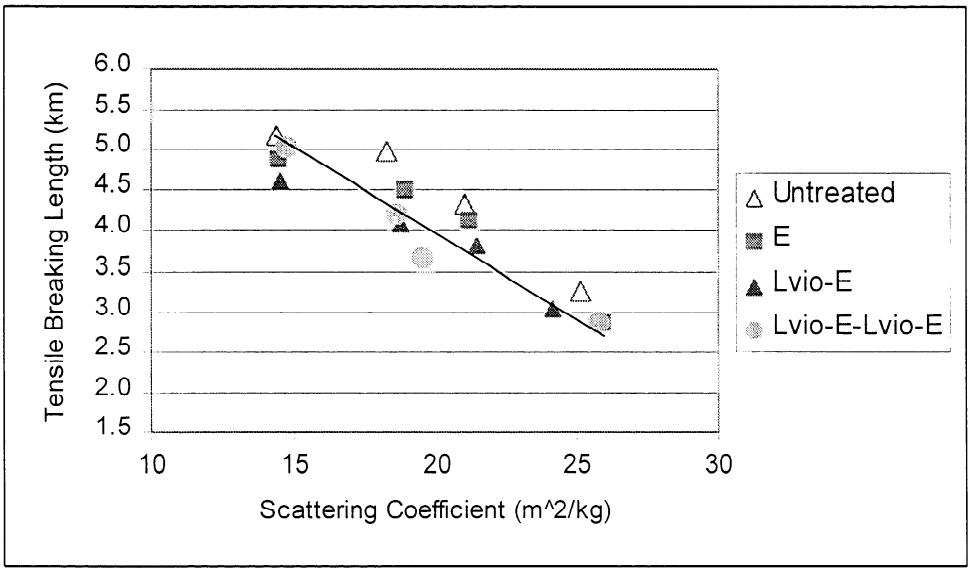

Figure 83: Tensile breaking length versus scattering coefficient for Lvio-E and Lvio-E-Lvio-E treated fully bleached pulp (FBP). There is no change in slope or intercept. Lvio-E: P-value for differences in slope = 0.68, P-value for differences in intercept = 0.16. Lvio-E-Lvio-E: P-value for differences in slope = 0.74, P-value for differences in intercept = 0.24.

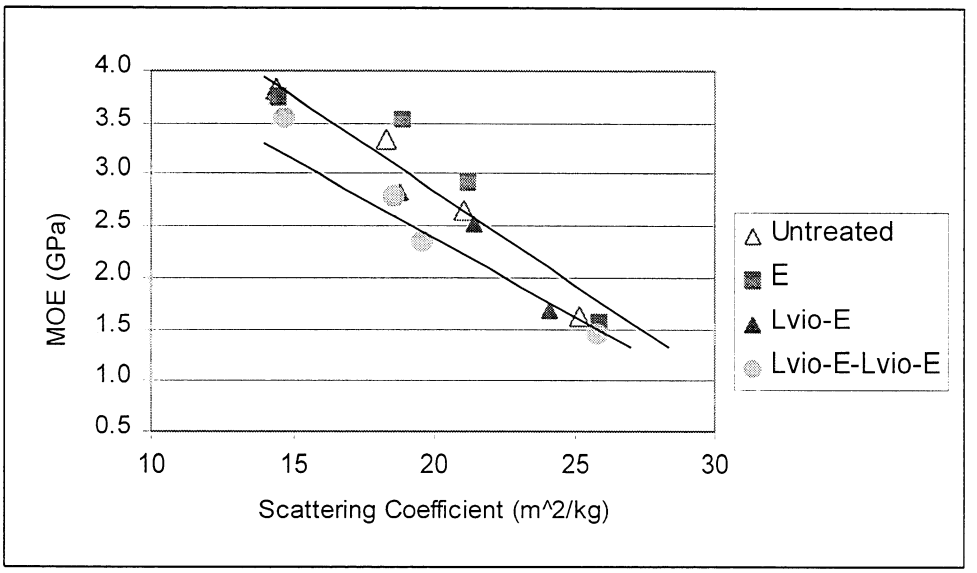

Figure 84: MOE versus scattering coefficient for Lvio-E and Lvio-E-Lvio-E treated fully bleached pulp (FBP). There is a decrease in intercept for the Lvio-E-Lvio-E treated pulp. Lvio-E: P-value for differences in slope = 0.83, P-value for differences in intercept = 0.23. Lvio-E-Lvio-E: P-value for differences in slope = 0.96, P-value for differences in intercept = 0.05. The upper line is for the alkaline extracted untreated pulp, and the lower line is for the Lvio-E-Lvio-E treated pulp.

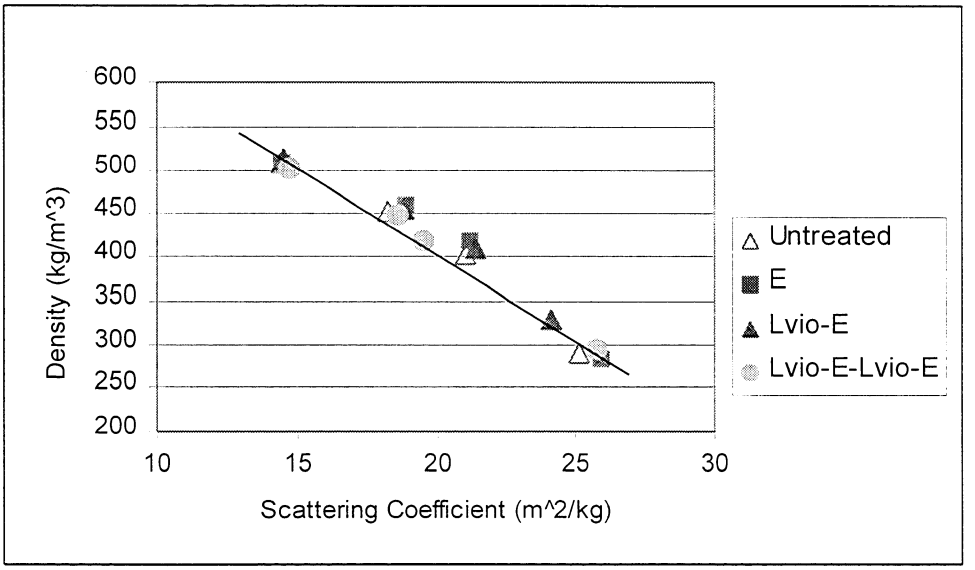

Figure 85: Density versus scattering coefficient for Lvio-E and Lvio-E-Lvio-E treated fully bleached pulp (FBP). There is no change in slope or intercept. Lvio-E: P-value for differences in slope = 0.77, P-value for differences in intercept = 0.93. Lvio-E-Lvio-E: P-value for differences in slope = 0.86, P-value for differences in intercept = 0.47.

Oxygen Delignification

Figures 86-89 show the physical properties of oxygen treated pulps. There was no change in Scott bond strength (Figure 86), no change in tensile breaking length (Figure 87), no change in MOE (Figure 88), and no change in density (Figure 89). Treating fully bleached pulp with oxygen appears to have no effect on paper physical properties when compared with an alkaline extraction stage. Again the kappa 23 pulp in Chapter 1 showed an increase in specific bond strength. The cause of this increase is most likely due to the removal of lignin.

It was expected that oxygen delignification would increase the Scott bond strength and tensile strength of the fully bleached pulp. A statistical analysis of covariance was done on the alkaline-extracted pulps. The results indicate that there was an increase in Scott bond strength (P-value for differences in slope = 0.26, P-value for differences in intercept = 0.035) for the alkaline-extracted pulp versus the untreated pulp. However, there was no change in tensile breaking length (P-value for differences in slope = 0.91, P-value for differences in intercept = 0.22), no change in MOE (P-value for differences in slope = 0.80, P-value for differences in intercept = 0.47), and no change in density (P-value for differences in slope = 0.93, P-value for differences in intercept = 0.47) for the alkaline-extracted pulp.

From this we can conclude that there was an increase in specific bond strength associated with the alkaline extraction, and that this change in specific bond strength was identical to the change in specific bond strength seen by both the oxygen and laccase-mediator treated pulps. This result is in agreement with the data presented by Barzyk (5) that indicated a similar strength improvement for both oxygen and alkaline-extracted pulps. The mechanism for this increase is

probably due to the removal of a thin layer of extractives or other contaminants from the fiber surface during the alkaline extraction stage.

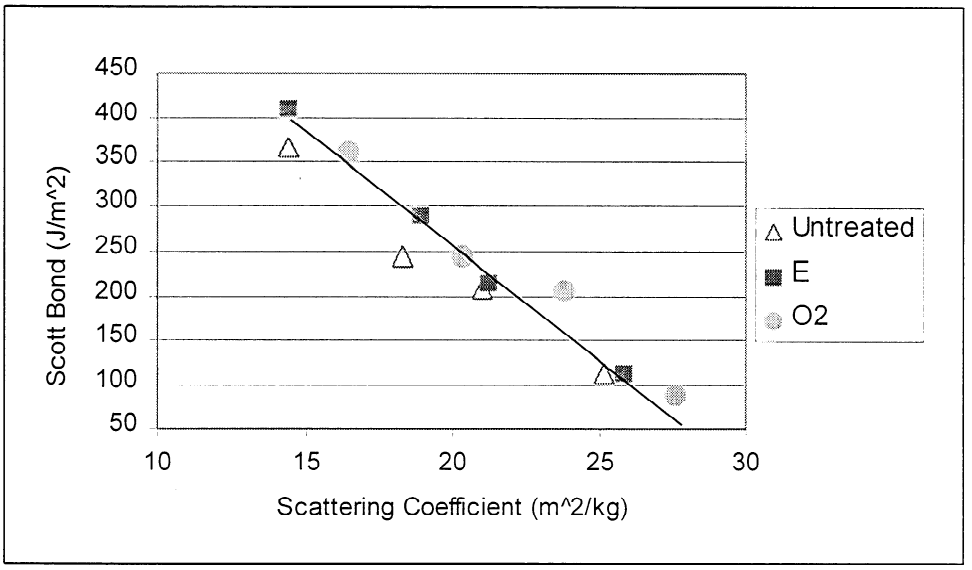

Figure 86: Scott bond strength versus scattering coefficient for O2 treated fully bleached pulp (FBP). There is no change in slope or intercept. For O2 versus E: P-value for differences in slope = 0.37, P-value for differences in intercept = 0.23.

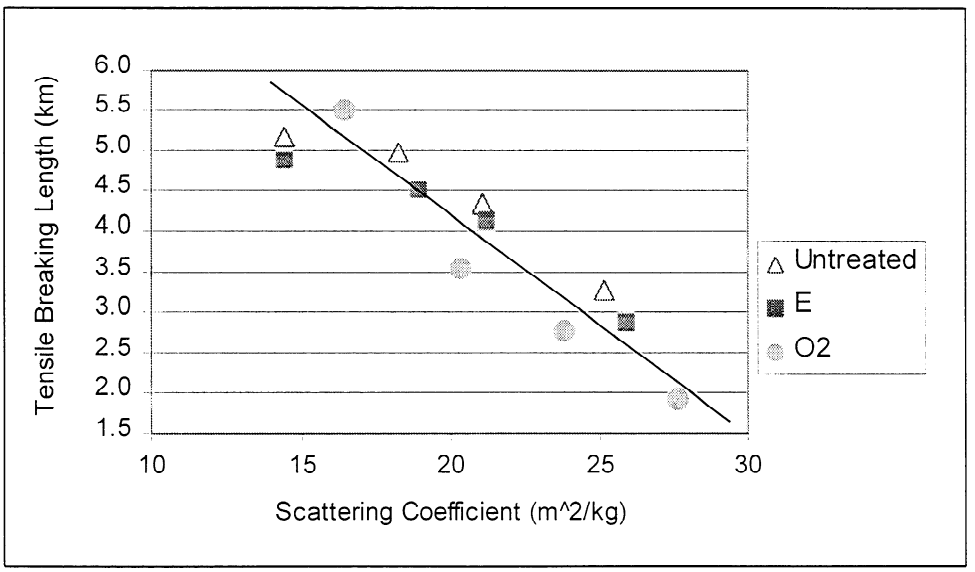

Figure 87: Tensile breaking length versus scattering coefficient for O2 treated fully bleached pulp (FBP). There is no change in slope or intercept. For O2 versus E: P-value for differences in slope = 0.092, P-value for differences in intercept = 0.61.

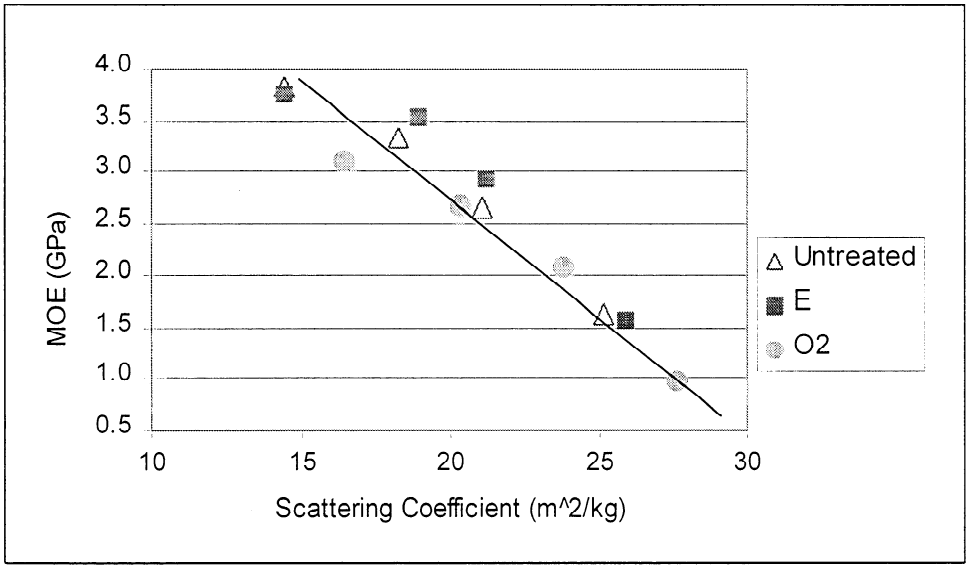

Figure 88: MOE versus scattering coefficient for O2 treated fully bleached pulp (FBP). There is no change in slope or intercept. For O2 versus E: P-value for differences in slope = 0.94, P-value for differences in intercept = 0.15.

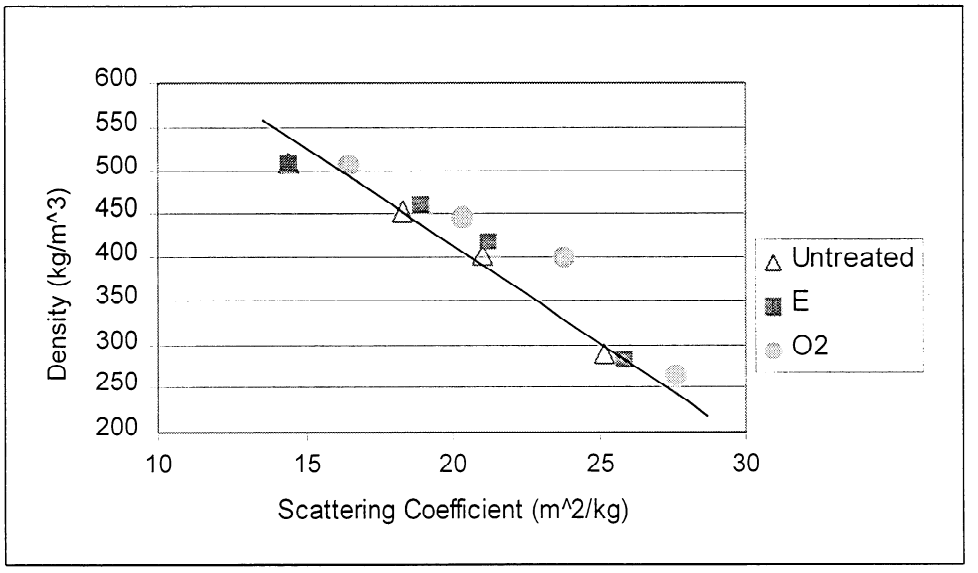

Figure 89: Density versus scattering coefficient for O2 treated fully bleached pulp (FBP). There is no change in slope or intercept. For O2 versus E: P-value for differences in slope = 0.86, P-value for differences in intercept = 0.23.

CONCLUSIONS

The treatment of fully bleached pulps finally allows us to identify any changes to the paper physical properties that may be caused by changes in the carbohydrate fraction of the pulp. It appears from previous work that the laccase-mediator systems are selective with respect to lignin, and no changes in the pulp physical properties were anticipated. The test results confirmed the selectivity of the laccase-mediator system with respect to lignin.

Treatment with laccase-mediator systems has little, if any, effect on paper physical properties for fully bleached pulp. The laccase-mediator systems appear to react almost exclusively with lignin. The slight drop in pulp viscosity and the decrease in brightness are the only indications that the laccase-mediator systems reacted at all with fully bleached pulp.

There was no increase in ESCA results for any of the treatments including oxygen and no increase in specific bond strength beyond that caused by alkaline extraction of the pulp.

Statistical analysis does show that the alkaline extraction stage produced an increase in specific bond strength, and that the laccase-mediator and oxygen treatments did not further increase the specific bond strength. This increase in specific bond strength is believed to be due to cleaning and removal of extractives and contaminates from the fiber surface.

THESIS CONCLUSIONS

Of the laccase-mediator systems studied, laccase-violuric acid was the best combination for delignification of loblolly pine kraft pulp. The laccase-violuric acid combination removed 35% of the lignin present in kappa 23 loblolly pine kraft pulp, versus 12 and 13% for laccase-HBT and laccase-NHAA.

The physical properties of Laccase-violuric acid delignified pulp were similar to those of oxygen delignified pulp, making the laccase violuric acid delignification system a promising alternative to oxygen delignification. The tensile breaking length of both kappa 23 and kappa 50 loblolly pine kraft pulp increased after treatment with laccase-violuric acid (followed by alkaline extraction) and oxygen delignification.

The laccase-mediator treatment was more selective with respect to lignin than oxygen delignification. The laccase-mediator delignification systems caused only a slight decrease in pulp viscosity for both kappa 23 and kappa 50 loblolly pine kraft pulps. Laccase-violuric acid was the most effective treatment for delignification, reducing the kappa number of kappa 23 loblolly pine kraft pulp by 35%, and decreasing the pulp viscosity by only 9%. In contrast, oxygen delignification reduced the kappa number by 22%, but decreased the pulp viscosity by 33%. Similar results for kappa 50 pulp showed a decrease in kappa number of 22%, and decreased the pulp viscosity by 2%. Oxygen delignification reduced the kappa of kappa 50 loblolly pine kraft pulp by 35%, but also reduced pulp viscosity by 40%. The oxygen delignification treatments resulted in a larger drop in pulp viscosity, which indicates increased carbohydrate degredation.

Carboxylic acid groups were introduced to the kappa 23 and kappa 50 pulps following laccase-mediator treatments. This increase was most profound for the kappa 50 pulps. When kappa 50 pulp was treated with laccase or any of the laccase-mediator systems with no alkaline extraction stage, there were large increases in surface carboxylic acid groups. The laccase-violuric acid treatment had the highest increase with over 150% increase in surface carboxylic acid content from 0.710 to 1.801 without a corresponding increase in Scott bond strength. The carboxylic acid groups appear to be located in the lignin pulp fraction. Surface carboxylic acid groups in lignin do not improve specific bond strength. It is clear from this work that the increase in specific bond strength associated with carboxylic acid groups (seen by other researchers) is due to the introduction of acid groups on the carbohydrate component of chemical pulp.

The laccase-mediator system introduces carboxylic acid groups to the lignin component of chemical pulp. In both kappa 23 and kappa 50 loblolly pine kraft pulps there were increases in surface and bulk carboxylic acid group content. No increases in surface or bulk carboxylic acid content were seen for the fully bleached Douglas fir industrial kraft pulp.

The laccase-mediator systems did not improve the physical properties of kappa 50 pulp, and in some cases decreased them. Kappa 50 handsheet strength properties were significantly decreased following treatment with laccase-mediator systems when no alkaline extraction stage was done to remove the reacted lignin material. A comparison between an acetone extracted and a non-acetone extracted kappa 50 pulp treated with laccase-violuric acid revealed that extractives played a role in decreasing the handsheet physical properties. By removing extractives from the pulp prior to Lvio treatment, the decrease in handsheet physical properties was eliminated. This suggested some reactivity between laccase, extractives and the pulp surface.

Delignification results from treating kappa 50 loblolly pine kraft pulp with dual laccase-violuric acid and dual oxygen delignification stages indicated that the laccase-violuric acid delignification system did not lose effectiveness in the second stage. Treatment of Kappa 50 pulp with dual laccase-violuric acid delignification stages resulted in first a 22% and then a 30% delignification. In contrast, oxygen delignification resulted in a 35% and then a 31% delignification. The laccase-violuric acid delignification reduced the kappa number by about twelve in each treatment. One possible reason for the difference between laccase-violuric acid and oxygen delignification is that oxygen reacts with and depletes the free phenolic groups in lignin. Laccase-violuric acid delignification does not.

Significant nitrogen contamination existed from both laccase and mediator sources. The nitrogen was bound to the lignin pulp fraction, and about half survives an alkaline extraction stage. Nitrogen contamination from enzyme and mediator sources was present in all cases where laccase or laccase-mediator systems came in contact with lignin containing pulp. The nitrogen contamination was highest for laccase-violuric acid treated pulp. About 50% of the nitrogen contamination was removed during an alkaline extraction stage. No nitrogen contamination was observed in the fully bleached Douglas fir industrial kraft pulps, indicating that the nitrogen contamination seen in lignin containing pulps was associated with the lignin pulp fraction.

Treatment of fully bleached Douglas fir industrial kraft pulp with the laccase-mediator systems did not introduce bulk or surface carboxylic acid groups, and did not increase specific bond strength above that of an alkaline extraction. Paper properties of oxygen delignified Douglas fir kraft industrial pulp were similar to those of the laccase-violuric acid treated and alkaline-extracted pulp.

Hydrogen bonding between hydroxyl groups on the surface of carbohydrates, both within and between fibers, is generally considered the mechanism of bonding in paper. The presence of carboxylic acid groups in carbohydrates is believed to enhance the ability of fibers to hydrogen bond, and improve specific bond strength. Previous research has focused on the addition of carboxylic acid groups to cellulose. This research focused on the changes in physical properties of pulps treated with laccase-mediator systems, including the effects of increased carboxylic acid content of lignin. The theory of bonding between carbohydrates does not predict the outcome of increasing the carboxylic acid content of lignin. In this research, the introduction of acid groups to lignin did not result in a change in physical properties. Additional results of this research do indicate that extractives on the surface of the pulp fibers can interfere with the development of bond strength between cellulose fibers. The removal of extractives material from the fiber surface resulted in an increase in specific bond strength. The extractives can be removed through alkaline extraction, allowing maximization of the natural bonding ability of the cellulose.

In summary, the studies in this thesis combine the areas of paper physics, biology and chemistry to determine the effects of state of the art laccase mediator delignification on fiber properties. As reviewed in the introduction, the goal of this research was to investigate the effects of laccase mediator delignification on fiber properties. As outlined in the experimental procedure section, carboxylic acid group topochemistry was determined using ESCA analysis and conductometric titration for different combinations of laccase and laccase plus mediator treated pulps. Using carefully controlled experimental protocol, the enzyme and mediator combinations were systematically varied, and the effects on carboxylic acid group location and bond strength determined. The results of this study are important in that they contribute to our understanding of the laccase mediator system, as well as the nature of bonding in paper.

RECOMMENDATIONS FOR FUTURE WORK

Future work on the effects of laccase mediator delignification should focus on mediator contamination and reacted mediator species contamination. Special attention should be given to the possibility of contamination with BT having a positive effect on specific bond strength. The role of extractives in laccase mediator delignification also warrants further study. The different mediators may react differently in the presence or absence of extractives. The effect of small amounts of extractive material on the surface of the fiber should be investigated further.

Recent literature has indicated that acid groups in lignin have pKa = 5.5. The deionized water used in this study for handsheet formation had a pH of about 6. At this pH some of the acid groups were protonated. The protonated acid groups may play a different role in bonding than the dissociated or ion exchanged carboxylic acid groups. The role of acid groups in lignin should be investigated by forming handsheets at higher pH and testing their strength properties.

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LITERATURE CITED:

1. Call, H. Patent: Multicomponent Bleaching System #WO 94/29425 Germany, 1994.

2. Amann, M.; "The Lignozyme Process Coming Closer to the Mill" ISWPC, 1997, Montreal.

3. Sealey, J. E. II; "Fundamental Investigation into Oxidoreductase Enzymatic Bleaching Systems" IPST Dissertation, Atlanta, GA (1998).

4. Scallan, A. M.; Tigerstrom, A. C. "Swelling and Elasticity of the Cell Walls of Pulp Fibers" Journal of Pulp and Paper Science 18(5):188-192 (September 1992).

5. Barzyk, D.; "Topochemical and Performance Aspects of Fiber Oxidation" Dissertation, IPST, July 1996.

6. Page, D. H.; "A Theory of the Tensile Strength of Paper" Tappi 52(4):674-681(1969).

7. Page, D. H.; Seth, R.S.; De Grace, J. H. "The Elastic Modulus of Paper" Tappi (9):99(1976).

8. Swanson, J.W.; Steber, A.J; "Fiber Surface Area and Bonded Area" Tappi 42(12):986 (December 1959).

9. Rundlof, M.; Bristow, J. A.; "A Note Concerning the Interaction Between Light Scattering Coefficient and Light Absorption in the Application of the Kubelka Munk Equations" Journal of Pulp and Paper Science, 23(5), p220-223 (1997).

10. Koukoulas, A. A.; Jordan, B. D.; "Effect of Strong Absorption on the Kubelka-Munk Scattering Coefficient" Journal of Pulp and Paper Science, 23(5), p224-231 (1997).

11. Koukoulas, A. A.; Jordan, B. D.; "Should the Kubelka-Munk Scattering Coefficient be Independent of Absorption" Pulp and Paper Reports PPR 818 Paprican (1990).

12. Olf, H. G.; "Correspondences Between the Kubelka-Munk and Stokes Model of Strongly Light Scattering Materials Part II: Implications" Tappi Journal, p159-163 (1989).

13. Skowronski, J.; Bichard, W.; "Fiber to Fiber Bonds in Paper. Part 1. Measurement of Bond Strength and Specific Bond Strength" Journal of Pulp and Paper Science, 13(5):j165-j169(1987).

14. Stratton, R.A. "Characterization of Fiber-Fiber Bond Strength from Paper Properties" 1991 International Paper Physics Conference: 561-577. Kona, Hawaii, September 22-26.

15. Koubaa, A; Koran, Z.; "Measure of the Internal Bond Strength of Paper/Board" Tappi 78(3):103 (1995).

16. Cole, B.J.W. et al. "Bond Strength of Oxygen Delignified Hardwood Kraft Pulps" 1996 Tappi Pulping Conference 481-489.

17. Mann, R.W.; Baum, G.A.; Habeger, C.C.; "Determination of All Nine Orthotropic Elastic Constants for Machine Made Paper" Tappi 63(2):163 (1980).

18. Laivins, G.V.; Scallan A.M.: "The Mechanism of Hornification of Wood Pulps" Paprican PPR 997, February, 1993.

19. Sjostrom, E. Wood Chemistry. Academic Press, New York, 1981.

20. Casey, Pulp and Paper Chemistry and Chemical Technology, Third Edition, Volume 2, 915-963 (1974).

21. Scallan, A. M.; Tigerstrom, A. C.; "Swelling and Elasticity of the Cell Walls of Pulp Fibers" Journal of Pulp and Paper Science 18(5):188-192 (September 1992).

22. Sjostrom, E.; "The Origin of Charge on Cellulosic Fibers" Nordic Pulp and Paper Research Journal 2:90-93 (1989).

23. Scallen, A.M.; Gringon, J.; "The Effect of Acidic Groups on the Swelling of Pulps, a Review" Tappi, 66(11):73-75 (1983).

24. Stenius, P.; Laine, J.; "Studies of Cellulose Surfaces by Titration and ESCA" Applied Surface Science 75, p 213-219, (1994).

25. Laine, J.; Lovegren, L.; Stenius, P.; "Potentiometric Titration of Unbleached Kraft Cellulose Fiber Surfaces" Colloids, Durf. A 88, p 277-287 (1994).

26. Laine, J.; "Effects of ECF and TCF Bleaching on the Charge Properties of Kraft Pulp" Paperi Ja Puu 79:8, p551-559 (1997).

27. Scallan, A.M.; Grignon, J.; "The Effects of Cations on Pulp and Paper Properties" Svensk Papperstidning nr 7:40-47 (1979).

28. Kobayashi, Y.; Matsuo, R. "Manufacture and Physical Properties of Alginate Fiber Papers as an Analysis and Model of Cellulose Fiber Papers" Journal of Applied Polymer Science 31(6): 1735-1747 (May 1986).

29. Lindstrom, T.; Soremark, C.; "Interaction Between Lignosulphonates and Simple Metal Ions" 50th Colloid Surface Science Symposium, San Juan, Puerto Rico, Volume 5: 217-230 (June 21-25, 1976).

30. Abubakr, S. M.; Hrutfiord, B. F.; McKean, W. T.; Reichert, T. W.; "Mechanism of Retention of Metal Cations in Linerboard and Never Dried Pulps" 1996 Recycling Symposium Proceedings (TAPPI): 43-50 (March 5, 1996).

31. Ohlsson, A.; Rydin, S.; "Washing of Pulps. Part 2. The Sorption of Na, Mg, Ca on Kraft Pulp" Svensk Papperstidning 78(15): 549-552 (October 1975).

32. Evens, B. E.; "The Effect of Process Water on Paper Properties" Paper Technology and Industry 22(3): 99-102 (April 1981).

33. Cohen, W.E.; Farrant, G. Watson, A.J.; "The Influence of Electrolytes on Pulp and Paper Properties" APPITA Proceedings: 72-104 (1949).

34. Evans, B.E.; "The Effects of Process Water on Paper Properties" Paper Technology and Industry, 22(3):99-102 (April, 1981).

35. Scallan, A.M.; Grignon, J.; "The Effects of pH and Temperature on the Swelling of Cellulose Gels" International Paper Physics Conference, Harrison Hot Springs, B.C.:53-62(September 17-19, 1979).

36. Strazdins, E.; "Surface Chemical Aspects of Polymer Retention" Papermakers Conference (TAPPI), Boston, Mass.: 59-65 (June 3-6, 1974).

37. Perkins, W. S.; Porter, J. J.; "A Study of the Thermodynamics of Sorption of Three Direct Dyes on Cellophane Film" Textile Research Journal 40(1): 81-88 (January 1970).

38. Ampulski, R. S.; Neal, C. W.; "The Effect of Inorganic Ions on the Adsorption and Ion Exchange of Kymmene 557H by Bleached Northern Softwood Kraft Pulp" Nordic Pulp and Paper Research Journal 4(2): 155-163 (July 1989).

39. Eguiazabal, J. I.; "Blends of Cellulose Acetate and an Aromatic Copolyester in the Presence of a Common Plasticizer" Cellulose Chemical Technology 19(4): 409-414 (July/August 1985).

40. Lindstrom, T.; Odberg, L.; Swerin, A.; "Deswelling of Hardwood Kraft Pulp Fibers by Cationic Polymers" Nordic Pulp and Paper Research Journal 5(4): 188-198 (December 1990).

41. Ampulski, R.S.; "The Influence of Fiber Surface Charge on Tensile Strength" Tappi Papermakers Conference Proceedings 9-16 (1985).

42. Erickson, E.; Sjostrom, E.; "The Influence of Acidic Groups on the Physical Properties of High Yield Pulps" Tappi 51(1):56-59(1968).

43. Sruunakki, A.; Heijnesson, A.; Buchert, J; Westermark, U.; Viikari, L.; "Effect of Pulp Surfaces on Enzyme-aided Bleaching of Kraft Pulps" JPPS 22:3, p91-96 (1996).

44. Laine, J.; Buchert, J.; Viikari, L.; Stenius, P.; "Characterization of Unbleached Kraft Pulps by Enzymatic Treatment, Potentiometric Titration, and Polyelectrolyte Titration" Holzforschung 50:3, p208-214 (1996).

45. Brasberg, S.; Thygeses, T.G.; Felby, C.; "Detection of Laccase Induced Modification of Beech and Spruce TMP Fibers by Luminescence Spectroscopy" 7th International Conference on Biotechnology in the Paper Industry, Vancouver, BC, Canada, p C123-126, June 16-19 (1998).

46. Poppius-Levlin, K.; Wang, W.; Tamminen, T.; Hortling, B.; Viikari, L.; niku-Paavola, M.L.; "Effects of Laccase/HBT Treatment on Pulp and Lignin Structures" ISWPC, Montreal, Canada, F3-1-F3-5 (1997).

47. Kantelinen, A.; Hortling, B.; Ranua, M,; Vikkari, L.; "Effects of Fungal and Enzymatic Treatments on Isolated Lignins and on Pulp Bleachability" Holzforschung 47:1, p29-35, (1993).

48. Katz, S.; Beatson, R. P.; Scallan, A. M. "The Determination of Strong and Weak Acidic Groups in Sulfite Pulps" Svensk Papperstidning 87(6):R48-52 (1994).

49. Horio, M.; Kobayashi, K.; Kondo, T.; "Investigation of Fine Structure of Freely Coagulated Viscose Fibers by Means of Optical and Electron Microscope" Textile Research Journal 17(5): 264-280 (May 1947).

50. Mitchell, K. A. R.; Ruddick, J. N. R.; Wong, P. C.; Yamamoto, K.; "X-Ray Photoelectron Spectroscopic Analysis of CCA (Chromated Copper Arsenate)-treated wood" Holzforschung, 47:6, p458-464, (1993).

51. Gray, D.G.; Takeyama, S.; "ESCA Study of the Chemisorption of Stearic Acid Vapor on Cellulose" Cellulose Chem. Technology 16:2, p133-142 (1982).

52. Hon, D. N.-S.; "Fourier Transform IR Spectroscopy and Electron Spectroscopy for Chemical Analysis (ESCA): Use in Study of Paper Documents" Advances in Chem. Ser. (ACS) no. 212, Chapter 19, p349-361 (1986).

53. Larsson, A.; Rigdahl, M.; Westerlund, B.; "Thermal Bonding of Cellulosic Networks with Polyethylene Fibers" Composite Systems from Natural and Synthetic Polymers, Elsevier, p83-96 (1986).

54. Nakamura, H.; Sawatari, A.; "Analysis of Functional Groups Formed on the Corona Treated Cellulose Fiber Sheet Surface by Means of Chemical Modification in Liquid-Phase-ESCA Technique" Journal Soc. Fiber Science Technology 49:6, p279-286 (1993).

55. Funato, N.; Nishiyama, S.; Sawatari, A.; "Analysis of the Functional Groups Formed on Corona Treated Cellulose Fiber Sheet Surface by Means of Chemical Modification in Gas Phase ESCA Technique" J. Soc. Fiber Sci. Technol. 49:7, p357-366 (1993).

56. Buchert, J.; Laine, J.; Stenius, P.; "Spectroscopic and Potentiometric Studies of Kraft Pulp Fibers" 48th APPITA Annual General Conference Proceedings, Paper 1B33: 109-115 (1994).

57. Carlsson, G.; Laine, J.; Stenius, P.; Strom, G.; "Surface Characterization of Unbleached Kraft Pulps by Means of ESCA" Cellulose, 1:2, p145-160 (1994).

58. Buchert, J.; Carlsson, G.; Harjunpaa, V.; Hausali, T.; Laine, J.; Strom, G.; Teleman, A.; Tenkanen, M.; Viikari, L.; Vuorinen, T.; "Effects of Pulping and Bleaching on the Surface Composition of Kraft Pulps" 8th International Symposium on Wood and Pulping Chemistry, June 6-9, Helsinki, Finland, p567-573 (1995).

59. Laine, J.; Stenius, P.; "Surface Properties of Bleached Kraft Pulp Fibers and Their Comparison with the Properties of Final Paper Products" 8th International Symposium on Wood and Pulping Chemistry, June 6-9, Helsinki, Finland, p589-596 (1995).

60. Aspler, J.; Zang, Y.; "Effects of Surface Binder Content on Print Density and Ink Receptivity of Coated Paper" Advanced Coating Fundamentals Symposium Proceedings, p 165-170 (1997).

61. Katz, S.; Gray, D. G.; "Solvent Extraction for the ESCA analysis of Paper" Svensk Papperstidning, 8, p226-228 (1980).

62. Istone, W. K. "X-Ray Photoelectron Spectroscopy" Surface Analysis of Paper, p 235-267, CRC press, New York, 1995.

63. Laine, J.; Stenius, P.; "Surface Characterization of Unbleached Kraft Pulps by Means of ESCA" Cellulose, 1, p145-160 (1994).

64. Garrett, R.; Grisham, C.; Chapters 11-13, Biochemistry, Saunders College Publishing, New York, p352-424 (1995).

65. Call, H. et al.; "State of the Art Enzymatic Bleaching Disclosure of a Breakthrough Process" Tappi Emerging Pulping and Bleaching Technologies Workshop. Durham NC, Mar 15-17, 1995.

66. Bajpai, P., "Biobleaching of Kraft Pulp" Biochemistry 27:319 (1992).

67. Kantelinen, A.; et al.; "Proposed Mechanism of Enzymatic Bleaching of Kraft Pulp Following Xylanase Treatment" Holzforschung 47:318 (1993).

68. Paice, M.; et al.; :Oxidative Bleaching Enxymes: The Next Generation?" International Pulping Conference, p211 (1994).

69. Lewis, N.; Yamamoto, E.; "Lignin: Occurrence, Biogenesis, and Biodegradation" Annual Review of Plant Physiology and Molecular Biology, 41:455-496 (1990).

70. Paice, M.; et al.; "Direct Biological Bleaching of Hardwood Kraft Pulp with the Fungus Coriolus Versicolor" Tappi Journal December: 91 (1992).

71. Erickson, K.; Blanchette, R.; Ander, P.; "Biodegradation of Lignin" Microbial and Enzymatic Degradation of Wood amd Wood Components. Springer Verlag, New York, p225-332 (1990).

72. Yaropolov, A.; et al.; "Laccase: Properties, Catalytic Mechanism, and Applicability" Applied Biochemistry and Biotechnology 49:257 (1994).

73. Spira-Solomon, D.; Allendorf, M.; Solomon, E.; "Low-Temperature Magnetic Circular Dichroism Studies of Native Laccase: Confirmation of a Trinuclear Copper Active Site" J. A. Chem. Soc. 108, 5318-5328 (1986).

74. Cole, J.; Tan, G.; Yang, E.; Hodgson, K.; Solomon E.; "Reactivity of the Laccase Trinuclear Copper Active Site with Dioxygen: An X-Ray Absorption Edge Study" J. Am. Chem. Soc. 112, 2243-2249 (1990).

75. Solomon, E.; Baldwin, M.; Lowery, M.; "Electronic Structures of Active Sites in Copper Proteins: Contributions to Reactivity" Cem. Rev. 92, 512-542 (1992).

76. Solomon, E.; "Coupled Binuclear Copper Proteins: Catalytic Mechanisms and Structure-Reactivity Correlations" Oxidases and Related Redox Systems Alan R. Liss Inc., 309-329 (1988).

77. Shin, W.; Sundaram, U.; Cole, J.; Zhang, H.; Hedman, B.; Hodgson, K.; Solomon E.; "Chemical and Spectroscopic Definition of the Peroxide-Level Intermediate in the Multicopper Oxidase: Relevance to the Catalytic Mechanism of Dioxygen Reduction to Water" J. Am. Chem. Soc. 118, 3202-3215 (1996).

78. Dean J.; Eriksson, K.; "Laccase and the Deposition of Lignin in Vascular Plants" Holzforschung 48(suppl.):21 (1994).

79. Potthast, A.; Rosenau, T.; Chen, C.; Gratzl, J.; "Selective Enzymatic Oxidation of Aromatic Methyl Groups to Aldehydes" J. Org. Chem. 60, 4320-4321 (1995).

80. Kawai et al.; "Aromatic Cleavage of 4,6-di-t-butylguiacol, a Phenolic Model Compound, by Laccase of *Coriolis versicolor*" FEB 236(2): 309-311 (August 1988).

81. Arcand, L.; Archibald, F.; "Direct De-Chlorination of Chlorophenolic Compounds by Laccase from Trametes (Coriolus) versicolor" Enzyme Microbial Tech. 13:194 (March 1991).

82. Bourbonnais, R.; Paice, M.; "Demethylation and Delignification of Kraft Pulp by Trametes versicolor Laccase in the presence of 2,2'-azinobis(3-ethylbenzthiazoline-6-sulphonate)" Applied Microbiology and Biotechnology 36:823 (1992).

83. Bourbonnais, R.; Paice, M.; "Optimization of Kraft Pulp Delignification by Laccase and a Mediator" Submitted to Tappi, 1995.

84. Call, H. et al.; "State of the Art Enzyme Bleaching and Disclosure of a Breakthrough Process" NCB (1994).

85. Sealey, J. E. II; Ragauskas, A.; "Fundamental investigation into the Chemical Mechanisms Involved in Laccase Mediator Biobleaching" ISWPC, Montreal, Canada, P F1-1- F1-4 (1997).

86. Tamminen, T.; Elder, T.; Wang, W.; Poppius-Levlin, K.; "The Action Mechanism of Laccase/1-Hydroxybenzotriazole System on Pulp Residual Lignin" 7th International Conference on Biotechnology, Vancouver, BC, A107-A110 (1998).

87. Bourbonnais, R.; Paice, M. G.; Leech, D.; Rochefort, D.; "Laccase/Mediator Bleaching of Kraft Pulps: Identification of Mediator Active Species and Their role in Oxidation of Residual Lignin Subunits" 7th International Conference on Biotechnology, Vancouver, BC, A103-A106 (1998).

88. Amann, M.; "The Lignozyme Process, Coming Closer to the Mill" ISWPC, Montreal, Canada, p F4-1-F4-4 (1997).

89. Pfaller, R.; Amann, M.; Freudenreich, J.; "Analysis of Laccase and Mediator Interactions in the LMS" 7th International conference on Biotechnology, Vancouver, BC, A99-A102 (1998).

90. Eachus, S.; "Atmospheric Pressure Oxygen Bleaching" Tappi Journal 58(9):151-154 (September 1975).

91. McDonough, T. J.; Reed, K. R.; "The Effect of Residual Lignin Carboxyl and other Acid Groups on Oxygen Bleachability of Kraft Pulp" TAPPI Pulping Conference, p657-673 (1991).

92. Gellerstedt, G.; Lindfors, E.L.; "Hydrophilic Groups in Lignin after Oxygen Bleaching" Tappi Journal, p119-122 (1987).

93. Sun, Y.; Argyropoulos, D. S.; "Fundamentals of High-Pressure Oxygen and Low-Pressure Oxygen-Peroxide (Eop) Delignification of Softwood and Hardwood Kraft Pulps: A Comparison" JPPS 21:6, pJ185-190 (1995).

94. Gratzl, J. S.; "Reactions of Polysaccharides and Lignins in Bleaching with Oxygen and Related Species" Tappi Oxygen Delignification Symposium, Toronto, Canada, p1-21 (1990).

95. Durso, D.; "Carbohydrate Reactions in Alkaline Pulping Processes" Chemistry of Delignification with Oxygen, Ozone, and Peroxides Symposium, Raleigh, NC, p 35-42 (1975).

96. Eriksson, K.; Blanchette, R.; Ander, P.; "Biodegredation of Lignin" Microbial and Enzymatic Degredation of Wood and Wood Components Springer Verlag, New York p225-332 (1990)

97. Oxley, P.; Adger, B.; Sasse, M.; Forth, M.; "N-Acetyl-N-Phenylhydroxylamine Via Catalytic Transfer Hydrogenation of Nitrobenzine Using Hydrazine and Rhodium on Carbon" Org. Syn. 67, 187-191 (1989).

98. Baker,W.; Sabapathy,K.; Vibat, M.; Lonergan,G.; "Laccase Catalyzes Formation of an Indamine Dye Between 3-methyl-2-benzothiazolinone Hydrazone and 3 dimethylaminobenzoic Acid" Enzyme and Microbial Technology 18, 90-94 (1996).

99. Viikari, L.; Karkki, T.; Niku-Paavola, M.; Buchert, J.; Poppius-Levlin, K.; "Oxidative Enzymes for Fiber Modification" 7th International Conference on Biotechnology in the Pulp and Paper Industry, Vancouver, BC, pA121-A124 (1998).

100 Hassingbow, J.; Lawther, J.; Felby, C.; "Influence of Extractives on Enzymatic Catalyzed Bonding of Norway Spruce TMP Fibers" 7th International Conference on Biotechnology in the Pulp and Paper Industry, Vancouver, BC, pA125-A128 (1998).

101. Laine, J.; Stenius, P.; "Effects of charge on the Fiber and Paper Properties of Bleached Industrial Kraft Pulps" Paperi Ja Puu, 79:4 p257-266, (1997).

102. Robert, D.; Szadeczki, M.; Lachenal, D.; "Chemical Characteristics of Lignins Extracted from Softwood TMP after O3 and ClO2 Treatment" Submitted August 1998.

103. Vasudevan, B.; Panchapakesan, B.; Gratzl, J.S.; Holmborn, B.; Tappi pulping conference, volume 3, p517-523, (1987).

104. Wong, K.; Anderson, K.; "The Mediator on LMS Modifies Handsheet Properties of High Kappa Kraft Pulps" 7th International Conference on Biotechnology in the Pulp and Paper Industry, pB211-B214 (1998).

105. Boa, W.; o'Malley, D.; Whetten, R.; Sederoff, R.; "A Laccase Association with Lignification in Loblolly Pine Xylem" Science 260:30, p672-674 (1993).

106. Okusa, K.; Miyakoshi, T.; Chen, C.; "Comparative Studies on Dehydrogenative Polymerization of Coniferyl Alcohol by Laccases and Peroxidases" Holz., 50:1, p15-20 (1996).

107. Devenyns, J.; Detroz, R.; Renders, A.; "Enhanced P Stages for ECF and TCF Bleaching" International Pulping Conference, p 295-302 (1996).

108. Devenyns, J.; Detroz, R.; Renders, A.; "Enhanced Two Stage Oxygen Delignification" 5th International Conference on New and Available Tech., Stockholm, Sweden, p778-789 (1996)

109. Johansson, E.; Suckling, I.; Ede, R.; "The Effect of O and OO Delignification on the Structure of Residual Lignin from Pinus radiata Pulp" ISWPC, pJ1-1-J1-4 (1997).

110 Greenwood, B.; "Oxygen Delignification Stage Design and Performance – A Kamyr Perspective" Tappi Bleach Plant Operations Short Course, p63-80 (1990).

111. Kersten, J,; Kalyanaraman, B.; Hammel, E.; Reinhammar, B.; "Comparison of Lignin Peroxidase, Horseradish Peroxidase and Laccase in the Oxidation of Methoxybenzenes" Biochem. J. 268:475 (1990).

113. Lundquist, K.; Kristersson, P.; "Exhaustive Laccase-Catalyzed Oxidation of a Lignin Model Compound (Vanillyl Glycol) Produces Methanol and Polymeric Quinoid Products" Biochem. J. 229:277 (1985).

114. Ishihara, T.; Ishihara, M.; "Estimation of Orthoquinone Structure and Methanol Formed from Lignin Models by Fungal Laccase" Mokuzai Gakkaishi 21:5, p323-325 (1975).

115. TAPPI 222 om-88, *Tappi Test Methods*, Atlanta, Tappi Press, 1992.

116. Kaar, W.; Brink, D.; "Simplified Analysis of Acid Insoluble Lignin" J. of Wood Chemistry and Technology, 11(4), 465-477 (1991).

117. TAPPI 249 cm-85, *Tappi Test Methods*, Atlanta, Tappi Press, 1992.

118. Kaar, W.; Cool, L.; Merriman, M.; "The Complete Analysis of Wood Polysaccharides Using HPCL" J. of Wood Chemistry and Technology, 11(4), 447-463 (1991).

119. Bjorklund, J.; Back, E.; Tufvesson, M.; "A Review of Kraft Pulp Deresingation and Pitch Problems" Papermaking Raw Materials, Mechanical Engineering Publications, London, Vol. 2, p729-760 (1985).

120. Strom, G.; Stenius, P.; Lindstrom, M.; Odberg, L.; "Surface Chemical Aspects of the Behavior of Soaps in Pulp Washing" Applied Surface Science, 75, p213-219 (1990).

121. El-Osta, M.; Wellwood, R.; Butters, R.; "An Improved X-Ray Technique for Measuring Microfibril Angle of Coniferous Wood" Wood Science. 25(2): 164-174 (1972).

122. Dence, C.; Reeve, D.; Pulp Bleaching, Principles and Practice, TAPPI PRESS, p215-239, Altanta, GA, (1996).

123. Freudenreich, J.; Amann, M.; Fritz-Langhals, E.; Stohrer, J.; "Understanding the Lignozyme Process", ISWPC, p71-76 (1997).

APPENDIX A: raw physical property data and standard deviations.

Chapter 1: raw data with standard deviations.

Sample	Wet Pressing Pressure (PSI)	Scattering Coefficient (m^2/kg)	st. dev.	Tensile Brk. Length (km)	st. dev.	Internal Scott Bond (J.m^2)	st. dev.	Tensile MOE (GPa)	st. dev.	Density (kg/m^3)	st. dev.
E	5	16.74	0.19	3.01	0.12	76	4.36	1.58	0.07	383	5.48
	50	15.29	0.04	3.78	0.10	111	5.83	2.52	0.06	517	17.58
	100	14.41	0.36	3.77	0.15	128	4.70	2.83	0.07	566	11.81
	500	13.86	0.42	3.88	0.18	127	5.83	2.87	0.07	585	7.80
Lhbt-E	5	16.98	0.37	3.30	0.17	79	3.52	1.64	0.04	386	4.56
	50	14.89	0.63	4.00	0.26	125	3.82	2.62	0.21	534	9.46
	100	13.33	0.36	3.95	0.19	127	5.44	2.65	0.12	571	11.24
	500	14.16	0.55	4.02	0.28	130	7.19	2.72	0.13	578	10.07
Lnhaa-E	5	16.79	0.52	3.03	0.25	79	5.48	1.43	0.16	373	6.27
	50	14.30	0.50	4.24	0.13	161	6.37	2.58	0.10	540	8.10
	100	13.73	0.42	4.33	0.21	162	6.37	2.63	0.16	568	15.07
	500	13.69	0.20	3.99	0.22	136	8.33	2.52	0.20	613	12.95
Lvio-E	5	16.20	0.35	3.64	0.16	93	2.82	1.71	0.10	400	5.05
	50	14.19	0.38	4.55	0.13	152	10.23	2.73	0.06	557	8.29
	100	13.11	0.13	4.66	0.15	169	9.11	2.86	0.15	596	12.59
	500	12.99	0.26	4.72	0.18	169	7.06	3.00	0.18	635	7.80
O2 , 1.5% NaOH	5	17.82	0.59	3.16	0.28	80	4.04	1.47	0.19	390	11.13
	50	15.23	0.27	4.14	0.22	136	11.76	2.44	0.10	534	5.85
	100	13.70	0.34	4.35	0.15	156	6.58	2.68	0.12	581	11.66

Chapter 2: raw data and standard deviations.

Sample	Wet Pressing Pressure (PSI)	Scattering Coefficient (m^2/kg)	st. dev.	Tensile Brk. Length (km)	st. dev.	Internal Scott Bond (J.m^2)	st. dev.	Tensile MOE (GPa)	st. dev.	Density (kg/m^3)	st. dev.
Untreated	5.00	17.27	0.29	3.02	0.10	70	7.22	1.66	0.07	385	9.13
	50.00	15.20	0.25	3.59	0.17	111	5.02	2.58	0.16	519	10.62
	100.00	14.87	0.28	3.65	0.19	131	7.78	2.64	0.16	559	13.46
	500.00	14.76	0.20	3.61	0.17	124	11.22	2.69	0.17	579	9.21
Lhbt	5.00	17.44	0.17	3.18	0.25	94	4.84	1.52	0.24	391	6.76
	50.00	14.87	0.48	3.91	0.27	138	7.34	2.51	0.06	541	7.76
	100.00	14.09	0.15	4.32	0.35	158	12.52	2.86	0.06	586	9.22
	500.00	14.55	0.14	4.14	0.09	147	11.79	2.86	0.06	582	17.71
Lnhaa	5.00	16.68	0.42	2.09	0.13	56	2.30	1.07	0.15	328	10.80
	50.00	14.61	0.13	2.94	0.19	90	8.92	1.99	0.20	488	16.45
	100.00	13.26	0.18	3.42	0.07	130	21.91	2.35	0.16	535	22.96
	500.00	14.54	0.25	2.88	0.23	117	4.04	2.08	0.24	569	23.95
Lvio	5.00	17.16	0.23	3.24	0.24	84	6.81	1.70	0.19	400	6.12
	50.00	14.47	0.21	4.45	0.09	136	12.47	2.59	0.11	540	13.34
	100.00	14.31	0.25	4.44	0.12	161	12.75	2.78	0.21	587	13.34
	500.00	13.71	0.33	4.35	0.18	162	8.54	2.70	0.07	630	12.48

Chapter 3: raw data and standard deviations.

Sample	Wet Pressing Pressure (PSI)	Scattering Coefficient (m^2/kg)	st. dev.	Tensile Brk. Length (km)	st. dev.	Internal Scott Bond (J.m^2)	st. dev.	Tensile MOE (GPa)	st. dev.	Density (kg/m^3)	st. dev.
Untreated	5	0.21	8.67	3.21	0.16	76	4.46	1.53	0.08	379	6.39
	50	0.61	7.52	3.97	0.15	132	6.97	2.41	0.19	532	6.91
	100	0.53	6.92	4.30	0.22	140	10.78	2.70	0.12	582	11.85
	500	0.36	7.11	3.88	0.21	140	20.03	2.50	0.16	610	15.39
L	5	0.97	8.67	2.14	0.16	57	2.97	1.00	0.15	324	6.41
	50	0.46	7.47	3.31	0.25	107	7.92	2.13	0.23	497	13.44
	100	0.71	6.93	3.47	0.22	124	8.59	2.38	0.16	538	15.15
	500	0.46	5.76	4.10	0.12	170	9.79	2.99	0.32	640	15.35
Lhbt	5	0.50	8.62	2.18	0.10	55	3.12	1.09	0.09	304	6.75
	50	0.45	7.36	3.46	0.18	102	7.06	2.32	0.10	490	10.24
	100	0.65	7.12	3.72	0.19	134	10.98	2.53	0.03	531	15.73
	500	0.20	6.91	3.19	0.31	86	4.04	2.45	0.16	533	13.07
Lhbt-E	5	0.41	8.59	1.97	0.24	57	2.57	1.01	0.15	309	8.49
	50	0.50	7.35	3.33	0.30	95	5.94	2.21	0.03	468	5.84
	100	0.25	7.01	3.42	0.36	118	9.04	2.44	0.23	527	18.19
	500	0.69	6.88	3.12	0.32	102	8.74	2.43	0.08	531	12.15
Lnhaa	5	0.41	8.67	2.21	0.18	60	3.19	1.02	0.15	314	6.66
	50	0.32	7.39	3.30	0.21	105	4.56	2.08	0.17	485	11.70
	100	0.31	6.95	3.55	0.08	128	1.49	2.22	0.10	538	17.61
	500	0.41	6.97	3.29	0.26	109	6.37	2.27	0.16	588	14.51
Lvio	5	0.33	8.48	2.42	0.13	61	2.97	1.12	0.13	334	12.54
	50	0.38	7.47	3.44	0.23	105	3.76	2.10	0.14	483	11.81
	100	0.29	6.94	3.83	0.24	129	5.27	2.30	0.11	529	13.93
	500	0.28	7.11	3.07	0.11	111	13.57	2.17	0.17	583	11.94

Chapter 4: raw data and standard deviations.

Sample	Wet Pressing Pressure (PSI)	Scattering Coefficient (m^2/kg)	st. dev.	Tensile Brk. Length (km)	st. dev.	Internal Scott Bond (J.m^2)	st. dev.	Tensile MOE (GPa)	st. dev.	Density (kg/m^3)	st. dev.
Untreated	5	16.97	0.07	1.33	0.04	42	1.05	0.78	0.12	185	3.93
	50	15.53	0.21	2.12	0.17	51	8.56	1.57	0.24	297	3.58
	100	14.01	0.22	2.66	0.12	104	12.16	2.01	0.20	352	1.00
	500	11.67	0.27	2.96	0.24	196	8.45	2.42	0.43	417	5.81
Lvio-E	5	15.88	0.35	1.72	0.15	29	17.33	0.88	0.13	198	2.04
	50	14.42	0.30	2.53	0.13	74	14.63	1.59	0.20	299	6.51
	100	12.90	0.16	3.46	0.24	115	9.29	2.24	0.24	346	28.87
	500	10.82	0.27	4.12	0.42	215	14.40	2.94	0.32	425	10.21
Lvio-E-Lvio-E	5	16.33	0.18	1.94	0.19	42	8.93	0.94	0.23	207	4.78
	50	14.43	0.43	2.95	0.20	87	7.04	1.89	0.23	319	9.72
	100	13.20	0.17	3.34	0.22	109	6.69	2.28	0.30	360	8.85
	500	11.32	0.20	3.80	0.26	194	11.90	2.76	0.39	427	4.40
O2	5	17.20	0.44	1.81	0.11	50	16.29	0.95	0.18	213	5.56
	50	15.19	0.53	2.52	0.14	95	10.32	1.75	0.30	328	8.52
	100	13.76	0.14	3.19	0.17	122	4.08	2.27	0.22	375	6.08
	500	10.96	0.23	3.82	0.25	224	11.00	2.80	0.42	440	4.06
O2-O2	5	16.36	0.23	2.28	0.26	62	6.72	1.33	0.17	263	4.29
	50	15.05	0.73	2.55	0.27	127	18.60	1.90	0.35	340	6.11
	100	12.86	0.11	3.07	0.16	234	9.66	2.30	0.27	376	7.19
	500	11.97	0.33	3.35	0.26	232	19.95	2.41	0.37	422	10.47

Chapter 5: raw data and standard deviations.

Sample	Wet Pressing Pressure (PSI)	Scattering Coefficient (m^2/kg)	st. dev.	Tensile Brk. Length (km)	st. dev.	Internal Scott Bond (J.m^2)	st. dev.	Tensile MOE (GPa)	st. dev.	Density (kg/m^3)	st. dev.
Untreated	5	16.97	0.07	1.33	0.04	42	1.05	0.78	0.12	185	3.93
	50	15.53	0.21	2.12	0.17	51	8.56	1.57	0.24	297	3.58
	100	14.01	0.22	2.66	0.12	104	12.16	2.01	0.20	352	1.00
	500	11.67	0.27	2.96	0.24	196	8.45	2.42	0.43	417	5.81
A-Untreated	5	16.75	0.29	1.70	0.16	37	8.43	0.97	0.26	224	5.75
	50	16.16	0.06	2.19	0.18	41	4.78	1.42	0.19	295	5.40
	100	14.79	0.28	2.65	0.31	87	3.18	1.94	0.30	346	3.65
	500	12.19	0.12	3.25	0.23	193	13.02	2.50	0.21	418	5.22
Lvio	5	16.01	0.23	1.79	0.28	46	1.75	0.93	0.11	205	2.84
	50	14.77	0.43	2.77	0.23	82	11.08	1.68	0.25	302	7.70
	100	13.34	0.08	3.49	0.22	124	14.02	2.18	0.19	346	5.67
	500	11.36	0.24	4.12	0.20	221	5.46	2.64	0.36	412	5.90
Lvio-E	5	16.07	0.17	2.06	0.19	52	8.45	0.93	0.21	211	7.93
	50	14.18	0.11	2.79	0.16	90	3.75	1.80	0.24	313	4.15
	100	13.22	0.20	3.31	0.38	131	15.00	2.16	0.32	354	3.38
	500	10.91	0.21	3.82	0.25	221	13.78	2.73	0.27	419	6.79

Chapter 6: raw data and standard deviations.

Sample	Wet Pressing Pressure (PSI)	Scattering Coefficient (m^2/kg)	st. dev.	Tensile Brk. Length (km)	st. dev.	Internal Scott Bond (J.m^2)	st. dev.	Tensile MOE (GPa)	st. dev.	Density (kg/m^3)	st. dev.
Untreated	5	25.15	0.29	3.27	0.21	114	4.82	1.64	0.15	289	4.13
	50	21.05	0.18	4.33	0.28	208	13.36	2.66	0.12	402	8.15
	100	18.27	0.34	4.97	0.19	245	7.61	3.34	0.14	452	8.25
	500	14.41	0.29	5.18	0.34	366	23.44	3.83	0.23	507	13.47
E	5	25.88	0.29	2.87	0.39	113	15.49	1.58	0.22	282	8.23
	50	21.20	0.40	4.15	0.24	214	2.00	2.93	0.34	419	7.90
	100	18.89	0.13	4.52	0.45	289	7.45	3.53	0.29	461	10.55
	500	14.44	0.26	4.88	0.42	411	8.71	3.74	0.20	509	13.52
Lhbt-E	5	24.41	0.45	3.54	0.23	164	16.18	1.93	0.12	349	15.82
	50	21.92	0.21	4.30	0.16	230	20.84	2.71	0.17	414	5.28
	100	18.01	0.58	4.44	0.26	274	6.62	3.04	0.19	449	8.04
	500	14.34	0.19	4.95	0.30	404	17.85	3.55	0.20	504	8.98
Lnhaa-E	5	24.88	0.14	3.47	0.13	177	16.92	1.94	0.10	347	7.82
	50	21.24	0.23	4.06	0.18	246	19.34	2.64	0.17	418	8.20
	100	19.23	0.12	4.29	0.46	286	15.14	2.84	0.12	449	10.60
	500	14.65	0.45	4.71	0.44	392	13.76	3.77	0.20	513	4.79
Lvio-E	5	24.13	0.77	3.05	0.20	139	5.81	1.68	0.09	330	8.33
	50	21.43	0.95	3.82	0.37	217	13.39	2.52	0.17	409	13.16
	100	18.80	0.49	4.10	0.24	268	32.49	2.84	0.21	454	4.71
	500	14.49	0.11	4.61	0.28	NA	NA	NA	NA	513	3.61
Lvio-E-Lvio-E	5	25.78	0.68	2.87	0.17	136	12.77	1.44	0.10	294	1.84
	50	19.55	1.62	3.67	0.37	247	20.32	2.34	0.17	417	15.94
	100	18.59	0.52	4.21	0.31	307	32.46	2.79	0.17	447	6.60
	500	14.71	0.79	5.03	0.41	426	37.39	3.55	0.21	502	17.66
O2	5	27.64	0.44	1.91	0.12	89	1.87	0.97	0.08	265	5.97
	50	23.82	0.25	2.78	0.36	207	8.68	2.07	0.16	400	3.16
	100	20.37	0.21	3.55	0.20	245	15.74	2.68	0.31	446	5.51
	500	16.45	0.41	5.49	0.69	362	17.42	3.10	0.24	507	14.86

APPENDIX B: Comparison between SKP50-1 and SKP50-2

Comparison between SKP50-1 (handsheets made at Weyerhaeuser) and SKP50-2 (handsheets made at IPST).

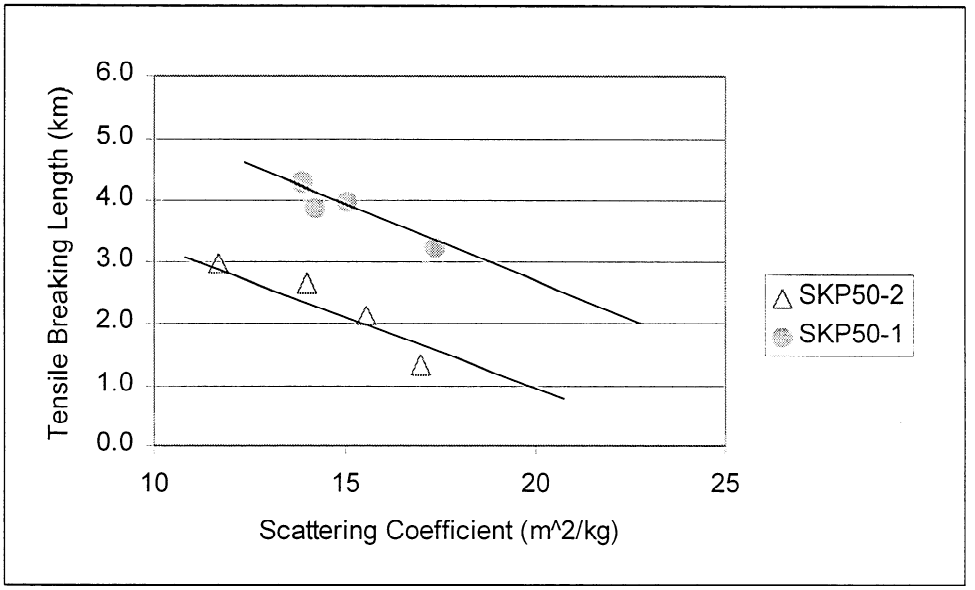


Figure 90: Tensile breaking length versus scattering coefficient for kappa 50 SKP50-1 and SKP50-2. P-value for differences in slope = 0.81. P-value for differences in intercept = 0.000086.

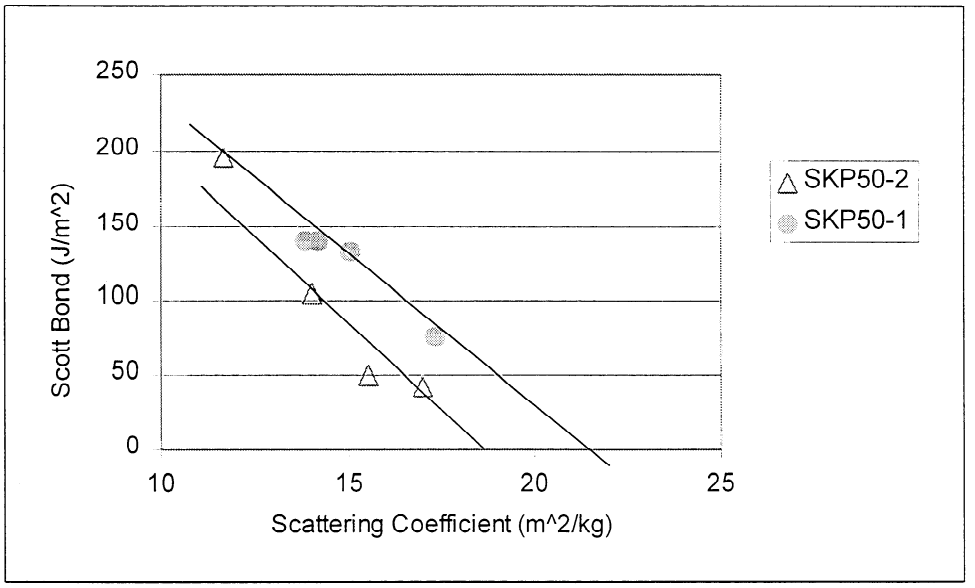


Figure 91: Scott bond strength versus scattering coefficient for kappa 50 SKP50-1 and SKP50-2. P-value for differences in slope = 0.194. P-value for differences in intercept = 0.028.

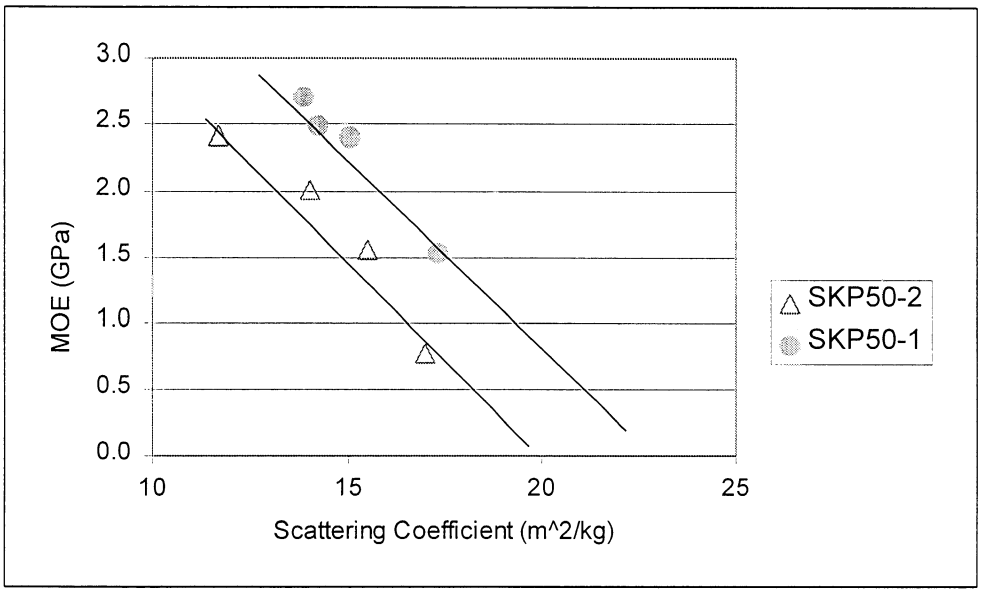


Figure 92: MOE versus scattering coefficient for kappa 50 SKP50-1 and SKP50-2. P-value for differences in slope = 0.739. P-value for differences in intercept = 0.00117.

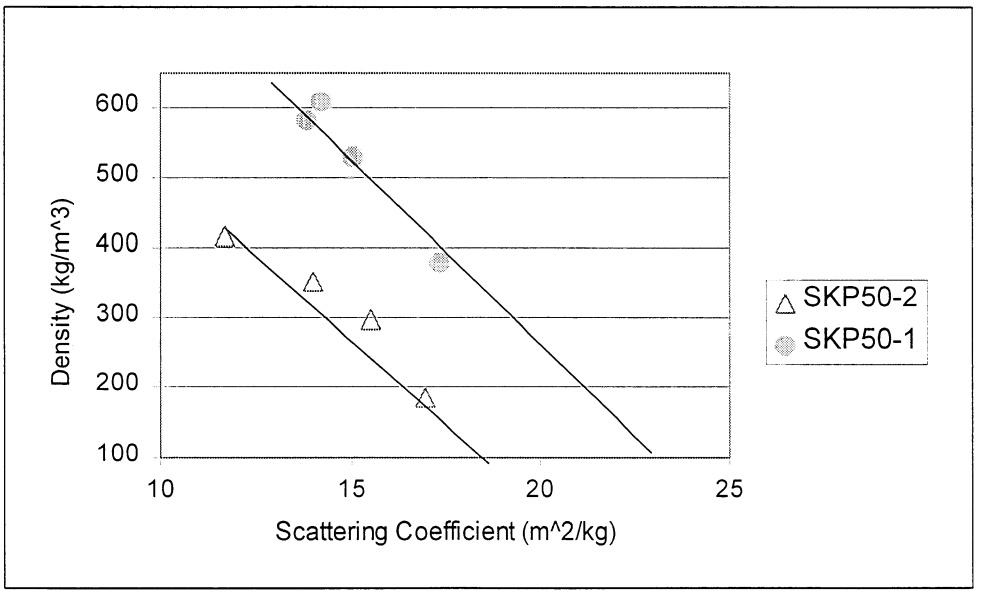


Figure 93: Density versus scattering coefficient for kappa 50 SKP50-1 and SKP50-2. P-value for differences in slope = 0.154. P-value for differences in intercept = 0.000177.

APPENDIX C: Equations used in statistical analysis

Statistics equations:

For analysis of variance:

$Y = b_o + b_1X_1 + b_2X_2 + b_{12}X_1 X_2$

Where	Y	Y variable (strength such as Scott bond)
	b_o	Intercept of line
	b_1	Slope of line
	X_1	X variable (Scattering coefficient)
	b_2	If significant indicates displacement of line
	X_2	Dummy Variable
	b_{12}	If significant indicates nonparallelism

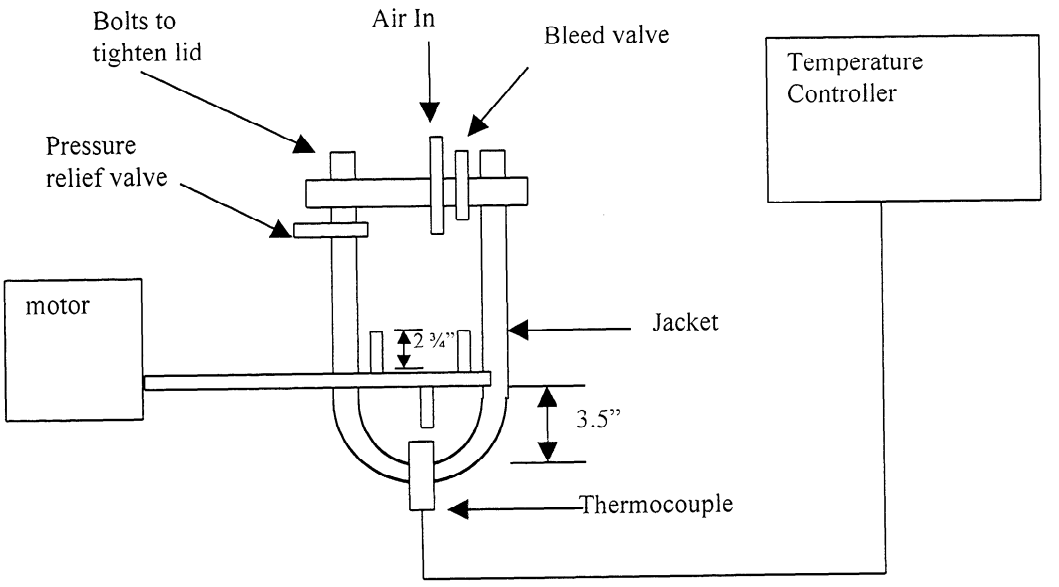
The hypothesis in the analysis of variance test is that b_2 and b_{12} are zero.

For Calculating pooled standard deviations:

$$S_p = \sqrt{\frac{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2 + ... + (n_{20} - 1)S_{20}^2 +}{(n_1 - 1) + (n_2 - 1) + ... + (n_{20} - 1)}}$$

where	S_p	Pooled standard deviation
	$n_1..n_{20}$	number of observations in each standard deviation
	$S_1..S_{20}$	standard deviations

APPENDIX D: Diagram of peg mixer



Volume: 4 L
Motor Speed: 100 RPM